The Effect of Sorting Wheat or Barley, Based on the Predicted Crude Protein Content, on Physical Characteristics, Feed Processing Characteristics and Nutrient Digestibility

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ABSTRACT

Variability in the physiochemical profile of cereal grains represents a challenge for the livestock industry. Currently, nutrient values are based on sample averages, ignoring the variation between individual seeds. These experiments were designed to determine if: (1) fractions obtained by an instrument calibrated to separate individual kernels based on predicted crude protein (CP) content have different physical characteristics and/or differ in dry matter digestibility (DMD) and amino acid digestibility (AAD); (2) the grinding method and intensity differentially influences digestibility of each fraction; and (3) hydrothermal treatment effects differ for the individual fractions. The BoMill TriQ (TriQ), which employs near infrared transmittance spectroscopy (NIT), was used to separate individual kernels based on predicted CP content.

In the first study, the TriQ was used to sort six independent sources of wheat into ten fractions each. A minimum of 100 kernels from each fraction were randomly selected and used to obtain measurements of length, width, height, area, DGM, perimeter, sphericity, colour (HunterLab), and mass. HunterLab was used to determine L (100 white to 0 black) a (-a green to +a red) b (-b blue to +b yellow). Data were analyzed as a complete randomized design (CRD) using Proc Mixed procedure of SAS 9.4 with the fixed effect being fraction. Physical characteristics were not different among fractions ($P > 0.10$), except that fractions with lower predicted CP content tended to have greater L* (54.12 vs. 50.95) based on the HunterLab calorimetric approach ($P < 0.10$).

In the second study, two fractions [predicted high CP (HCP) and low CP (LCP)] produced from five independent sources of feed grade wheat or barley, were compared to the unsorted (UNS) grain. Each fraction (UNS, HCP, and LCP) was ground through a 0.375-mm (coarse grind) or a 0.188-mm (fine grind) screen using a hammer mill or a roller mill to produce coarse and fine treatments. The UNS fraction was used to adjust the roller mill to produce ground samples with a similar processing index (w/v) relative to the hammer mill. *In vitro* DMD (using rumen inoculum; %), starch digestibility (%) and total gas production (TGP; mL) were determined after

a 12-h incubation. Data were analyzed independently by grain type including the effects of fraction, grinder type and severity of processing, and their interactions. A split plot design was used where main plot was fraction and the subplots were grinder type and severity of grinding. Significance was defined as $P < 0.05$ and a trend was defined as $P > 0.05$ and $P < 0.10$. The TGP (mL) and DMD (%) of barley ground using a hammer mill were greater than when processed using a roller mill ($P < 0.05$; 59.4 mL ± 2.0 mL and 41.8% ± 1.0%, respectively). A similar response was observed for wheat processed using either a hammer mill or a roller mill ($P < 0.05$; 63.8 mL ± 1.4 mL and 27.8% ± 1.5%, respectively). Increasing the severity of processing increased TGP (47.4 mL ± 1.96 mL vs 35.9 mL ± 1.98 mL), DMD ($P < 0.05$; 36.2 % ± 1.03 % vs 29.6 % ± 1.04 %) for barley and for wheat ($P < 0.05$; 48.9 mL ± 1.48 mL vs 42.7 mL ± 1.44 mL) and DMD ($P < 0.05$; 36.4% ± 0.82% vs 32.2% ± 0.80%). Sorting individual seeds based on predicted CP content did not affect physical characteristics, DMD, or TGP for either wheat or barley.

In the final experiment, the response of fractions to hydrothermal treatment on AAD was assessed. Eight wheat-based and eight barley-based treatment diets were used. These treatments followed a 2 × 2 × 2 factorial arrangement where the main factors were processing temperature (low vs. high temperature pelleting), fractions (LCP vs. HCP) and grain sources (two independent sources for each of wheat and barley). Additionally, a nitrogen-free diet was fed to estimate endogenous losses. Sixteen ileal cannulated pigs were fed the diets in six blocks, providing an $n = 6$ per treatment. Digestibility of amino acids for rations composed of barley had a fraction × temperature of processing interaction; the same was observed for wheat grain except for proline ($P = 0.27$), glycine ($P = 0.16$) and histidine ($P = 0.46$), while trends were observed for phenylalanine ($P = 0.07$), tyrosine ($P = 0.10$), isoleucine ($P = 0.07$), methionine ($P = 0.08$) and glutamic acid ($P = 0.08$). Most of the amino acids for wheat and barley did not exhibit differences between fractions in digestibility. This lack of difference for the majority of the parameters was attributed to the similarity in chemical composition between the fractions. The similarity between the fractions was attributed to the inability of the TriQ to separate a source into fractions that were different chemically.

The key findings of the experiments were that using NIT to sort on an individual seed basis for

predicted CP content did not result in chemical (starch or CP content) or physical variation. Additionally, DMD and TGP did not differ between the fractions produced by NIT. The current NIT technology was found to have limitations in its ability to differentiate kernels that may affect chemical, physical or processing traits.

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DEDICATION

To all the pigs that became my pets, the two cows that provided the rumen fluid, to my family for their patience in handling my frustrations and to my friends who were there whenever I needed them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAD	Amino acid digestibility
ADF	Acid detergent fiber
AOAC	Association of Official Agricultural Chemists
CFRC	Canadian Feed Research Center
CP	Crude protein
CRD	Completely randomized design
CV	Coefficient of variation
CWRS	Canadian Western Red Spring
CWSWS	Canadian Western Soft White Spring
DDGS	Distiller's dried grains with solubles
DE	Digestible energy
DGM	Geometric mean diameter
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DON	Deoxynivalenol
GP	Gas production
HCP	High crude protein
HPT	Homan pellet tester
HT	High temperature, pelleted at 85°C
LCP	Low crude protein
LT	Low temperature, pelleted at 70°C
ms	Milliseconds
NDF	Neutral detergent fiber
NIR(S)	Near infrared reflectance spectroscopy
NIT(S)	Near infrared transmittance spectroscopy
PDI	Pellet durability index
PDS	Prairie Diagnostic Services

PI	Processing index
PSC	Prairie Swine Centre
r^2	Coefficient of determination
SD	Standard deviation
S_{gw}	Standard deviation of geometric mean diameter
SECV	Standard error of cross validation
SEM	Standard error of the mean
SID	Standardized ileal digestibility
SKCS	Single kernel characterization system
SSP	Salt soluble protein
TGP	Total gas production
TKW	Thousand-kernel weight
TriQ	BoMill TriQ
UNS	Unsorted
WHC	Water hydration capacity

1 INTRODUCTION

Feed represents a major operational cost in cattle and swine production (Herd et al. 2003; Woyengo et al. 2014). Wheat and barley are the major grains incorporated into livestock rations in western Canada. Several empirical and mechanistic models have been developed to estimate the nutritional requirements for livestock under current and future management practices (Tedeschi et al. 2005). The models use average chemical composition values to allow an operator to recognize the potential outcome of using various ingredients and their inclusion levels on animal health and performance.

Estimates provided by these models have resulted in the development of methods to increase the efficiency of feed use by changing management practices and ensuring adequate nutrients, especially energy and protein, are provided (Fox et al. 2004). These models use average values for a batch of grain, where a batch of grain refers to grain purchased from one location and sampled and analyzed for nutrient content as a single entity. However, within a batch, protein and energy content can vary considerably, and the inability of a model to use this variation can result in inefficiency in livestock feeding or inconsistencies in the final product.

Similarly, previous research determining the nutritional value of a feed used ingredients from a bulk source, thus variability was presumed to represent average but these researchers did not take into account that the average may be skewed to the right or left. Few studies have attempted to determine the variability within lots or between individual kernels. The use of tools such as near infrared reflectance spectroscopy (NIR or NIRS), near infrared transmittance spectroscopy (NIT), imaging technology (colour sorters and camera-based technology) and gravitational tables makes it possible to sort for chemical (e.g. crude protein; CP) or physical properties (e.g. density) of feedstuffs. While the use of these technologies could produce grain fractions that are more consistent, it is not known if this extra step would improve net returns for the feed industry.

Research is required to determine:

1. Whether fractions produced by such technologies differ in chemical and physical

traits;

2. Whether fractions differ in particle size and particle size distribution resulting from grinding and rolling relative to the initial batch;
3. If fractions respond differently to hydrothermal treatment; and
4. Whether there is sufficient difference between fractions to impact animal performance.

The literature review which follows will examine variability within batches of wheat or barley separated by NIR and the potential for fractionation of grains using NIR.

2 LITERATURE REVIEW

Grain arriving at a feed mill often comes from multiple sources and may vary in quality. Currently, grain entering a terminal or a feed processing facility is sorted based on characteristics of the entire load average. Grains entering a mill are sampled and analyzed using either wet chemistry or near infrared reflectance spectroscopy (NIRS). Near infrared reflectance spectroscopy has lower accuracy and precision than wet chemistry (Blanco and Villarroya 2002); however, its efficiency and operational cost (Corson et al. 1999) make it a viable alternative in a commercial setting (Huang et al. 2008). Near infrared reflectance spectroscopy is nondestructive allowing it to be incorporated into an in-line system (Kelley et al. 2004a; Kelley et al. 2004b), and costs less per sample than wet chemistry (Corson et al. 1999; García and Cozzolino 2006). In-line NIR systems have been tested and developed for the pharmaceutical (Hailey et al. 1996; Sekulic et al. 1998), food and beverage (Huang et al. 2008), and petroleum industries (Alves et al. 2012). A limited number have been incorporated into commercial feed mills.

2.1 Grain Structure

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are the primary grains grown in western Canada. In North America, wheat is classified as hard or soft, referring to the energy required during grinding for flour production, while barley is generally referred to as malting or feed, which refers to the industry they are destined for. Globally, 2/3 of barley is grown for animal feed, 1/3 for malting and less than 2% is grown specifically for the food industry (Baik and Ullrich 2008), whereas most of the wheat is grown for the food industry (Rosenfelder et al. 2013; Gonzalez-Esteban 2018).

Wheat kernels grown in North America average 8 mm in length and weigh 35 mg (Hoseney 1994). However, the size and mass vary by cultivar (Fifield et al. 1945; Dziki and Laskowski 2005), growing environment (Dziki and Laskowski 2005) and the location of the kernel within the plant (Hoseney 1994). Wheat kernels are rounded on the dorsal side and have a crease that is longitudinal and covers the length of the ventral side (Figure 2.1). The wheat crease may contain debris that passes into the final product following grinding (Hoseney 1994).

The wheat kernel is composed of several layers (Figure 2.1). Wheat bran, composed

mainly of lignified cell wall which is resistant to digestion (Knudsen and Hansen 1991), comprises approximately 14.5% of a wheat kernel (Gwirtz 1998). The pericarp layer, which is 5% of the total mass of the bran is a lignified layer composed of arabinoxylans and cellulose (Knudsen and Hansen 1991; Evers and Millar 2002; Dziki and Laskowski 2006). The aleurone layer of the bran contains high levels of protein and lipids (Knudsen and Hansen 1991). The endosperm consists of two layers, the aleurone and the starchy endosperm wall. The aleurone layer is abundant in ash and CP and has a high level of enzymatic activity (Hoseney 1994). The cell walls of the starchy endosperm are composed of non-starch polysaccharides, e.g. arabinoxylans, ß-glucans, and hemicellulose (Antoine et al. 2003). The germ has no starch but is high in protein, sugars, oil, vitamin B and ash (Gwirtz 1998). The germ contains enzymes responsible for germination. Enzymes are present in several different layers within a wheat kernel (Rani et al. 2001). The sugars within the germ are mainly sucrose and raffinose (Hoseney 1994). The mechanical properties of wheat are due to the endosperm and bran layers (Dziki and Laskowski 2006) whereas the starch content accounts for most of the energy within the kernel (Gwirtz 1998).

In smaller wheat kernels, 80% of the kernel is starchy endosperm, 3.5% is germ (embryo and scutellum) and 15.5% is pericarp, testa and aleurone (Marshall et al. 1984b). In contrast, 83.5% of the kernel is starchy endosperm in large wheat kernels, 2.5% is germ (embryo and scutellum) and 14% is pericarp, testa and aleurone (Marshall et al. 1984b). This difference between small and large kernels may impact the final products after grinding. The lack of homogeneity will further reduce the consistency of the final product exiting the facility.

Barley grain has similar structural characteristics to wheat albeit with exceptions. The minor dimension of barley is between 2.5 and 2.9 mm with a 1000-kernel weight (TKW) ranging from 36 to 54 g (Andersson et al. 1999). A barley kernel also contains several layers. The husk (hull) of the barley grain consists of the lemma and palea and comprises 10 to 13% of the total kernel weight (Evers and Millar 2002). Hulless barley, where the hull is removed during threshing, has been developed (Boros et al. 1996). Like wheat, barley starchy endosperm is composed primarily of starch and the endosperm wall is composed of a protein matrix and β-D-glucan non-starch polysaccharides (Duffus et al. 1992; Hoseney 1994).

The starchy endosperm contains almost all the starch in both wheat and barley (Hoseney 1994; Evers and Millar 2002). It is composed of two different cell types: peripheral prismatic and central (Gwirtz 1998; Evers and Millar 2002). Peripheral prismatic and central cells differ in chemical composition and it is predicted that variation within these cells will result in differing particle size after grinding, subsequently affecting digestibility. Starch represents the greatest weight of a kernel for both barley and wheat and is the major energy for feed rations (Suileiman 1995). Starch characteristics (type of starch granules and granule size) vary between and within wheat (Zeng et al. 1997) and barley (Holtekjolen et al. 2006) varieties. Moreover, the environmental conditions where barley (Tester 1997) or wheat (Matsuki et al. 2003) is grown affect starch structure and composition (proportion of amylose to amylopectin can result in a change in three-dimensional structure), even within the same cultivar.

The variation in wheat shape or size within a sample can result in a reduction in grinding homogeneity (Bramble et al. 2002). Larger wheat kernels are harder and result in finer grinding products (Gaines et al. 1997). Variability in wheat kernel diameter depends on location within the head (spike) plant; kernels from the second floret were found to be larger and heavier than kernels from the other florets, and kernels from apical spikelets had lower kernel weight and lower CP than other kernels in the spike (Ali et al. 1969; Rawson and Evans 1970; Kirby 1974).

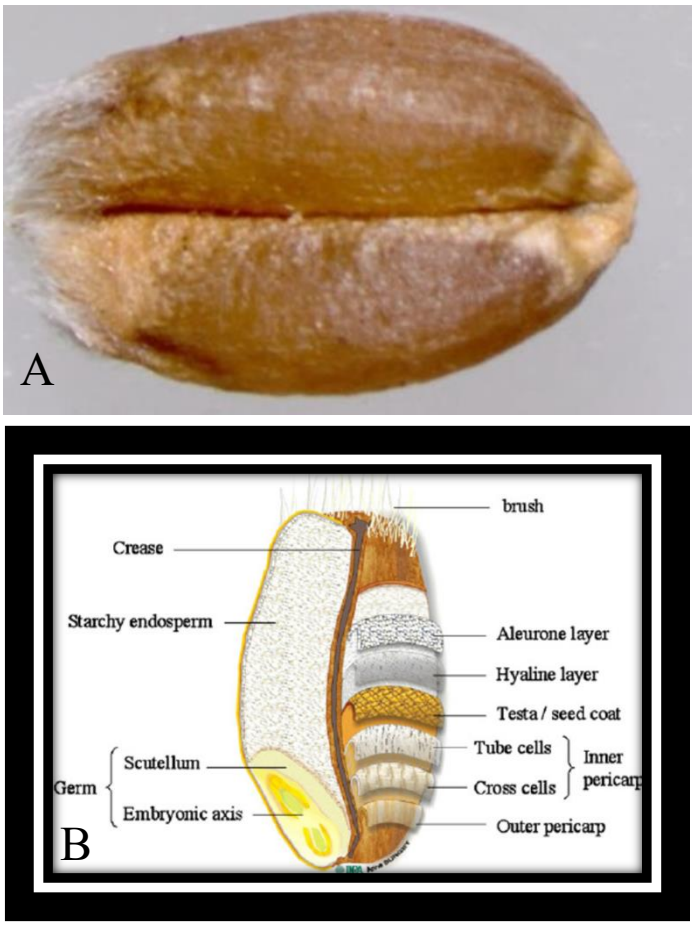

Figure 2.1. The structure of a wheat kernel: (A) photograph illustrating the location and depth of the crease and (B) the histological composition (adapted from Surget and Barron 2005).

2.1.1 Variation in Physical Characteristics

Test weight measures grain density by weighing grain of a specified volume, TKW measures the weight of 1000 kernels and bushel weight is a density of measurement that can either fill a volume of 36.37 L (Avery bushel weight) or 35.24 L (Winchester bushel weight). The physical traits of wheat or barley such as geometric mean diameter (DGM), weight of individual kernels and test weight are influenced by growing conditions (environmental and management practices) and variety (Khorasani et al. 2000).

For wheat, the quality of the grain is determined by weight, hardness, size, shape, vitreousness and colour (Crosbie et al. 1998; Al-Saleh and Brennan 2012). These traits are analyzed prior to grinding as they have the potential to affect flour and feed quality. For barley, traits such as kernel size, shape and weight, and consistency, are important (Blake et al. 2010).

Density is the weight of the sample per unit volume (Dobraszczyk et al. 2002). Zijlstra et al. (1999) examined several different wheat samples harvested in the Province of Saskatchewan in 1992 and found that density varied from 45 kg/hl to 77.6 kg/hl. These samples included 11 hard red spring wheat, four prairie spring wheat varieties and a durum wheat variety (Zijlstra et al. 1999). However, more than 40 years ago, Christison and Bell (1975) showed that the test weight of wheat and barley had no relationship with energy content, and density was a poor predictor (r^2=0.43) of digestible energy (DE) content. Zijlstra et al. (1999) found that crude protein (CP) and neutral detergent fibre (NDF) provided a more precise prediction of DE than did test weight (r^2 = 0.75 vs. r^2=0.43, respectively). Conversely, Fairbairn et al. (1999) found that the test weight of barley samples were significantly correlated to DE. Current commercial varieties may produce different results.

The barley endosperm contains mostly starch, the major energy source within the barley kernel (Jenner et al. 1991), but it may not comprise the greatest percent of total weight and thus may not be a major contributor to test weight (Hunt 1996). The importance of variation in density was demonstrated by work conducted by Peters and Katz (1962), where the kernel density of wheat varied from 1.29 to 1.41 g/cm^2, corrected to 12% moisture. The inability of the endosperm to have a profound impact on the test weight explains why there were no observed

changes in DE when different test weights of barley were compared. However, kernel length and width were negatively correlated with test weight (Schuler et al. 1994) and thus sorting based on test weight may result in an increase in kernel consistency; this was not investigated by Schuler et al. (1994).

Marshall et al. (1986b) showed that the test weight of wheat had a strong positive correlation to milling yield, with the fraction containing kernels larger than 2.8 mm diameter having the highest milling yield, relative to the fractions containing kernels less than 2.2 mm in diameter. Milling yield, defined as the amount of flour produced per unit volume of wheat, can be impacted by several factors, including the percent of endosperm, the size of the embryo, and the morphological characteristics of the seed coat, grain hardness, bulk density and crude fibre content (Marshall et al. 1986b). The correlation between density and milling yield for winter and spring wheat was poor (Hook 1984). The correlation between test weight and CP was low in soft wheat (Wilkins et al. 1993).

Fairbairn et al. (1999) used barrows to test 20 different barley samples and identified that most of the variation in energy content was accounted for by test weight and acid detergent fibre (ADF). Grimson et al. (1987) investigated three different barley varieties with different test weights and observed that test weight had no effect on dry matter (DM) intake or daily gain for finishing steers. Conversely, lambs fed heavy test weight barley had significantly greater gains than those fed light test weight barley (Yang et al. 2000).

A consistent moisture level is required for accurate test weight measurements (Christison and Bell 1975). Khorasani et al. (2000) measured TKW on 60 different cultivars of barley and determined that it ranged from 42.9 g/1000 kernel to 53.9 g/1000 kernel. Fairbairn et al. (1999) found a coefficient of determination (r^2) of 0.65 between TKW and DE for barley based on 20 cultivars. DE and ADF was negatively correlated and DE had a higher correlation to ADF than test weight (Fairbairn et al. 1999). Similarly, Bhatty et al. (1975) found that DE for wheat was negatively correlated with TKW, whereas DE for barley was positively correlated with TKW.

Unlike density, individual kernel shape can vary considerably and is due to genetic variation and thus the shape of individual kernels can be altered by genetic selection (Marshall et

al. 1984b). Fractionation, using a combination of kernel shape and density, can be accomplished with a gravity table providing an increase in product homogeneity (Siemens and Jones 2008). Gravity tables separate lighter kernels from heavier kernels by forcing an upward stream of air through the kernels as the kernels move down an inclined plane (Siemens and Jones 2008). Gravity tables can be used as tools to segregate grains based on physical density and have been developed to screen out impurities, based on size or density (Nielsen et al. 2003; Siemens and Jones 2008; Berghmans et al. 2010).

Tkachuk et al. (1990) used a specific gravity table (Spiroll Kipp Kelly Model SY 300., Winnipeg, Manitoba; capacity 580 kg/h) to sort five different Canadian red spring wheat samples with grades from number 1 to feed. Gravity tables separate kernels based on their density; high test weight fractions had larger kernels than low test weight fractions, and thus they are likely to have the largest endosperm (Tkachuk et al. 1990). The CP content varied between fractions (Tkachuk et al. 1990). Wilkins et al. (1993) used a gravity table to separate white wheat samples and observed a negative correlation between test weight and CP.

Using a gravity table, Siemens and Jones (2008) investigated the relationship between kernel density of soft white wheat and test weight, protein content and milling performance. It was found that when wheat kernels were separated by density, test weight, CP and milling performance were positively correlated to density, and more so than with the initial unsegregated sample. They concluded that an increase in the homogeneity of the sample improved the correlation between density and test weight, CP and milling performance. Commercially, the use of gravity tables has been limited for wheat, but it may be promising for barley if removing high CP fractions alters beer haze, which impacts quality (Siebert 1999).

Miller (2008) used a single kernel characterization system (SKCS) over 10,000 individual wheat kernels to look at the effect of farm, plot, spike, spikelet and floret position on physical characteristics and found that there were significant variations in hardness, diameter, weight, and moisture content between spikes. The SKCS (Perten Instruments, Springfield, IL) weighs individual kernels and then each kernel is crushed. The SKCS uses the energy required to crush the kernel to calculate the diameter, moisture and the hardness index of individual kernels

(Osborne and Anderssen 2003). Osborne and Anderssen (2003) indicated that SKCS can be used as a tool for genetic mapping, grain testing and screening of wheat.

Kernel plumpness is important in the malting industry and can play a crucial role in determining the quality of grain for feed. The higher the plumpness, the lower the percentage of hull and/or bran (Christison and Bell 1975). Plumpness can be determined by measuring the weight grain remaining on a 3.1-mm sieve after the sieve has been shaken for 2.5 minutes (Christison and Bell 1975).

Barley kernels with low plumpness have low starch and high protein and fibre content compared to kernels with higher plumpness (Hunt 1996). Plumpness is rarely measured in a feed mill, but test weight is, and feed producers may give a discount or premium based on test weight. Test weight and plumpness are both related to density (Spilde 1989). Uniformity of kernel plumpness is crucial for ruminants because kernels must be ground, and inconsistency can result in inconsistent crushing creating a greater proportion of fines which can cause digestive disorders (Edney et al. 1994). Barley kernel plumpness was greater for six row cultivars of barley than for two row cultivars (Bhatty et al. 1974). In this study, six row cultivars of barley had a greater number of seeds that were small when compared to the two row cultivars of barley (Bhatty et al. 1974). When shriveled wheat kernels, which have lower plumpness, were removed, test weight increased by an average of 36 kg/m^3 (Schuler et al. 1994). Schlau *et al.* (2013) ground light weight and heavy weight barley (based on TKW) under two different roller milling setting and found that TKW did not affect ruminal fermentation which can be attributed to using TKW rather than actual kernel size. Ahmad et al. (2010), on the other hand, sieved kernels prior to grinding; roller mill was adjusted for kernel size and it was found that using this approach did affect rumen fermentation. Elfverson et al. (1999) used an automated weight sorter that weighed individual kernels to sort five different barley cultivars into different weights and found that CP did not vary significantly between the different weights. Regnér (1995) showed that wheat with a higher TKW contained more CP content and ash compared to wheat with a lower TKW.

2.1.2 Variation in Chemical Composition

Chemical composition can vary between and within batches. The impact of such

variability has been studied; however, the work is older and limited work has been performed using current varieties and feeding systems.

2.1.2.1 Variation in Energy Content

Wheat and barley are the major sources of energy for monogastrics and ruminants in western Canada (Clark et al. 2009). There is a negative relationship between CP and energy content in these grains (Hunt 1996). Starch, the major storage carbohydrate in cereal grains, is composed of amylose, a linear molecule where D-glucose units are connected by α-(1,4) glycosidic links, and amylopectin, a polymer where D-glucose units are connected by α-(1,4) glycosidic linkage with branching at α-(1,6) glycosidic linkages (Hoseney 1994; Singh et al. 2010). Starch content can vary from 53 to 67% in barley (Per et al. 1985). Stein et al. (2016) found that the starch content of 77 samples of wheat averaged 57.6%, whereas Kim et al. (2005) revealed a range of 50.4% to 79.5% in wheat which would result in differing digestibility for pigs. Digestibility of grain is affected by the ratio of amylose to amylopectin (Gidley 2001; Singh et al. 2010). A waxy cereal is a cereal that contains a higher content of amylopectin, relative to a non-waxy grain (Ankrah et al. 1999). According to Baik and Ullrich (2008), amylose content of barley starch can vary from 0% to 5% in waxy barley, from 20 to 30% in normal barley, and may reach 45% in high-amylose barley varieties. This variation in amylose content could also affect particle size, particle shape and pellet characteristics generated by processing.

Starch digestibility is also affected by granule morphology, molecular structure, the degree of branching of amylopectin and the method of processing, especially hydrothermal processing (Singh et al. 2010). Ankrah et al. (1999) examined waxy and normal barley varieties within rations and found that pelleting at 75°C with 10% moisture added through steam increased starch digestibility by 17%, reduced digesta viscosity by 45%, and improved overall energy digestibility in broilers. Using the rumen *in-situ* nylon bag incubation technique, Khorasani et al. (2000) found that the starch content of barley was positively correlated to the soluble protein fraction, while the test weight was correlated to the degradable protein fraction and total CP content (Khorasani et al. 2000).

2.1.2.2 Variation in Protein Content

Cereals are an important protein source for livestock, but they are deficient in lysine and other essential amino acids (Shewry 2007). The importance of CP in wheat and barley plays a role in determining nutritional value, processing characteristics and overall cost; CP content can vary between varieties as well as within a variety. Amino acid composition plays an important role in determining the quality of wheat (Bramble et al. 2002) and total CP content can impact its market price and end use (Delwiche 1998). Barley CP content quality has been improved over the years by increasing the lysine content (Jood and Singh 2001).

Several researchers have investigated variability in protein type and quantity within wheat. Wheat protein can be divided into four major types based on solubility and functionality (Malik 2009). The four major types are albumins, prolamins, globulins and salt soluble proteins (SSP). Albumins are soluble in water and diluted buffers, whereas prolamins are soluble in salt solutions (Malik 2009). Albumins and globulins have higher lysine and methionine than prolamins and SSP (Lásztity 1984). Salt soluble proteins are sensitive to heat treatment (Odjo et al. 2012). Several different SSP proteins exist in wheat, including α-amylase inhibitor, peroxidase and non-specific lipid transfer protein (de Gregorio et al. 2009). Prolamins are soluble in 70 to 90% ethanol, whereas globulins are soluble in dilute acid or alkali (Malik 2009). Barber et al. (1988) indicated that, for barley, the major proteins are albumins and globulins, which can constitute up to 25% of the CP content and contribute up to 50% of the lysine. Other protein, such as glutelins and prolamins have also been isolated from barley (Linko et al. 1989). Goesaert et al. (2005) indicated that, for wheat, non-gluten protein is between 15 and 20% of total protein, whereas gluten protein represents 80 to 85% of the total.

Limited research indicates that the average CP content of individual kernels of wheat can vary by 6%, ranging from 15% to 18% (Levi and Anderson 1950), whereas barley can vary by 8 to 15% (Kirkman et al. 1982; Shewry 2007). Growing conditions impact CP quantity and quality within wheat. For example, grain yield is negatively correlated to wheat CP content (Terman et al. 1969) and CP content increased with higher environmental temperature during growth with high levels of nitrogen fertilizer application (Daniel and Triboi 2000). The proportion of ω-gliadins to total gliadins increased while γ-gliadins decreased with increasing temperature but the

ratio decreased with increasing nitrogen fertilizer (Daniel and Triboi 2000). Soft wheat varieties contain two proteins related to hardness, puroindoline-α and β, which interact with starch to impact the hardness of the kernel (Swan et al. 2006). Hard wheat has either a mutation or no puroindoline, resulting in the hard wheat texture (Morris 2002; Swan et al. 2006).

The SKCS can test each kernel for moisture, kernel size, weight and hardness (Osborne and Anderssen 2003). The SKCS assesses individual kernels by crushing them at a rate of two kernels per second with a toothed rotor and a crescent (Osborne and Anderssen 2003). Kernel diameter, moisture, weight and hardness index are determined using formulas derived by Martin et al. (1993) using standardized samples that are used to calibrate the SKCS (Osborne and Anderssen 2003). Gwirtz (1998) found a positive correlation between CP and SKCS hardness prediction. This positive relationship was observed between CP and prediction of hardness using NIRS (Pasha et al. 2006). Pomeranz et al. (1985) used NIRS to examine hardness for several wheat varieties obtained from around the world. It was found that environment affected the hardness to a lesser extent than did variety, and thus, genetics plays a major role in wheat hardness (Pomeranz et al. 1985). Unlike wheat, there is a lack of research on barley hardness.

2.2 Sorting of Grain Based on Single Kernel Characteristics

Separating grain into single kernels has been conducted using visual-based technology and NIRS. Visual-based technology is technology that is based on detecting visible light. Near infrared spectroscopy technology is based on detecting infrared wavelengths using either reflectance or transmittance technology. Separating grains on a single kernel basis has met with limited success due to several factors, some of which are discussed below.

2.2.1 Visual-Based Technology

Visual based technology is based on the detection of light in the visible range of the electromagnetic spectrum, with images being captured using one or several cameras. This technology can be used to detect differences in colour between kernels, but it is not capable of physically separating individual kernels. A colour sorter uses an air ejector and can remove kernels from the bulk sample, which results in the ejection of all unwanted kernels as well as some of the desired kernels as defined by the user (Berghmans et al. 2010). The use of ejectors

increases the number of kernels removed and sorted per second. While this can result in up to 15% of the desired grains entering the undesired fraction (Pasikatan and Dowell 2003), sorting capacity is improved, which is necessary for a commercial setting.

The use of colour sorting technology has been implemented in the grain industry to improve product consistency and remove contaminants. Colour sorters can be used to remove impurities (Pearson et al. 2008) such as ergot contaminated grain (Inamdar and Suresh 2014) or to separate red wheat from white wheat (Pasikatan and Dowell 2002). Delwiche et al. (2005) used an optical sorter to produce different fractions and found that sorting reduced deoxynivalenol (DON) contamination by up to 69%. Similarly, using an optical sorter (model CS-300, Satake Corp., Hiroshima Japan), Saito et al. (2009) found that the proportion of fusarium-damaged kernels could be reduced, which correlated to lower DON levels. These studies illustrate the potential value of a colour sorter to improve seed quality and consistency.

Colour sorters are used by wheat breeders to purify their breeding lines and for exporters to meet requirements for export (Pasikatan and Dowell 2003). Colour is an important determinant of market price in the export market (Melchor and Floyd 2002) and premiums may be provided if specific colour requirements are met. In some markets, this premium is only given if 90% or more of the wheat kernels display the required colour (Hou et al. 1998).

Colour sorters examine individual kernels. Additionally, image sorters can also examine individual kernels. Pearson et al. (2008) used an image sorter to separate red wheat kernels from white wheat kernels. This system used a camera and several mirrors to photograph individual wheat kernels from three sides, after which the information was analyzed by a computer. The computer, through a separate input, triggered air valves to allow uniquely coloured kernels to enter different containers (Pearson et al. 2008). While this system could accurately separate white kernels from red kernels at a rate of 30 kernels s^{-1}, this throughput is too low for a commercial setting. The use of colour sorters and image capture technology has been limited to wheat; information on barley or other grains is limited. Moreover, while barley colour varies from light yellow to purple, some barley varieties are resistant to discolouration (Baik and Ullrich 2008).

2.2.2 Near Infrared Reflectance Spectroscopy (NIRS)

Near infrared reflectance spectroscopy and near infrared transmittance spectroscopy (NIT) measure the reflectance and transmittance of light between 780 nm and 2500 nm of the electromagnetic spectrum. When light reaches an organic compound, it causes bending and stretching vibrations in -CH, -NH, -SH and –OH bonds (Corson et al. 1999; Gillon et al. 1999; Cen and He 2007). These vibrations and stretches are unique to each biological sample (Hacisalihoglu et al. 2010) and the spectrum of each sample can be described by the absorption and reflectance in terms of wavelength, height and width of each peak.

Unlike wet chemistry, NIRS is a secondary technology that requires calibrations. These calibrations are based on determinants acquired with wet chemistry. As a result, the accuracy of NIRS is limited by the accuracy of the wet chemistry method used (Geladi et al. 1985). The initial set of samples used to develop the calibration must represent the variation expected within the population (Park et al. 1998). Therefore, NIR will have errors related to wet chemistry and due to the instrument used to obtain the spectrum (Park et al. 1998; Nicolaī et al. 2007).

Advancements in fiber optics, computing power, detectors and filters, as well as reduction in the overall cost of purchase and maintenance, have made NIR instruments more widely available (Cozzolino et al. 2006) and portable (dos Santos et al. 2013). The energy of the electromagnetic wave that an NIR instrument uses penetrates a sample with declining intensity as it passes through the sample (Nicolaī et al. 2007). Penetration depth is the depth where light intensity declines by 1% from the initial intensity generated by the beam (Fraser et al. 2003). Unlike X-ray energy, the energy that penetrates by NIR is low energy and does not cause damage to the sample (McClure 2003; Pasquini 2003; Pellicer and del Carmen Bravo 2011).

The low energy and low penetration of NIS instruments makes NIRS sensitive to particle size. The smaller the particle, the greater the scatter, resulting in a lower peak height (Geladi et al. 1985; Hareland 1994; Pasikatan et al. 2002; Nicolaī et al. 2007) and distortion of the data. Additionally, changes in particle size, shape and packing density can affect the scattering of the energy passing through or leaving a particle (Pasikatan et al. 2001). Methods have been developed to correct for such distortion, including multiplicative scatter correction (Geladi et al.

1985; Bull 1991; Pasikatan et al. 2002) which takes the average spectrum and adjusts individual spectra accordingly (Naes et al. 1990).

Sensitivity of NIRS or NITS has been reviewed by several authors (Pasikatan *et al.* 2001; Reich 2005). Near infrared reflectance spectroscopy has had some success in determining particle size of powders with high accuracy (O'Neil et al. 1998). To reduce the effect of particle size in the prediction of the chemical composition of a grain sample, the calibration must use samples ground with a high-speed hammer mill or an impeller type of grinder (Williams 1979). Such measures will improve the accuracy and precision of the instrument and the calibration developed.

The International Organization for Standardization (ISO) has defined accuracy as the closeness of agreement between test and reference results, whereas precision is defined as closeness or agreement between independent test results under the same conditions (ISO 1994). The accuracy of NIRS is dependent on the calibration used and can be determined by cross-validation. Cross validation is obtained when a subsample of the samples used to develop the calibration are used to evaluate the accuracy of the model (Bokobza 1998; Cen and He 2007). The calibration equation that is proposed to predict the constituent in question is used to generate the coefficient of determination (r^2) (Williams and Norris 2001) and the standard error of calibration (SECV) can also be calculated (Landau et al. 2006). The standard error of cross validation is the difference in the variability between the predicted value, using the calibration Equation, and the reference value. To calculate the SECV, the equation must be applied sequentially to subsets of data from the calibration set. These errors must be calculated for each calibration model. The accuracy and precision of NIR will be limited by the accuracy and precision of the initial wet chemistry used in the development of the calibration (Corson et al. 1999).

Calibrations developed for NIR predictions of feed quality include predictions of moisture, hardness, protein, oil, granularity, insect infestation and grain yield (McClure 2003). While the ash content for wheat rations had an r^2 of 0.97 (Garnsworthy et al. 2000) and for barley grain an r^2 of 0.91 (Sohn et al. 2008), the determination of mineral content using NIR has

been limited. Several NIR calibrations have been developed for the measurement of CP in wheat. Single kernel CP content for wheat using NIR is based on the spectrum between 850 and 1048 nm (Delwiche 1995) with the highest accuracy for single kernel CP in the 1100 to 1400 nm range. If an instrument was developed to predict just CP, only a narrow spectrum would be required, and the overall cost of the instrument would be reduced.

Williams (1979) developed calibrations for prediction of CP in wheat using NIRS with high accuracy and precision. Additionally, Williams et al. (1984) used NIRS to predict lysine, threonine, tryptophan and methionine, also with high accuracy and precision. Mutlu et al. (2011) developed NIRS calibrations for wheat and found r^2 values of 0.95 and 0.83 for CP and water absorption capacity, respectively. Previous work has shown that the DGM was correlated to CP content (Williams and Thompson 1978), which could explain the inaccuracies with NIR where particle size distribution between samples resulted in different severity of grinding.

Near infrared reflectance spectroscopy calibrations have been developed for forages to predict dry matter intake (DMI), CP content, ADF and *in vitro* organic matter digestibility (Garcia and Cozzolino 2006). Kays et al. (2005) developed calibrations to predict total dietary fibre (TDF) for ground barley using NIR with wavelengths ranging from 1104 to 2494 nm with an r^2 of 0.96. Coefficients of determination of 0.82 were achieved for unground barley using NIT (Kays et al. 2005). Garnsworthy et al. (2000) used 160 samples of wheat, representing over 20 varieties, to generate NIRS calibrations for chemical profile and nutritive and agronomic characteristics. The r^2 values for predicting DM, CP, ash and oil ranged from 0.90 to 0.98, whereas the r^2 for starch was only 0.78 (Garnsworthy et al. 2000). The reason for the lower r^2 for predicting starch was not determined within this study but it could have been due to inaccuracy in wet chemistry in determining starch, variability in starch content or the complex chemical characteristics of starch relative to the other variables assessed. Garnsworthy et al. (2000) showed that NIRS calibrations produced for wheat to predict energy and nitrogen digestibility in pigs had low r^2 values of 0.17 and 0.22, respectively.

The physical characteristics of wheat have been predicted with high accuracy using NIRS. Most of these studies used ground grain. Hardness, a trait that can influence further processing

and which millers use to indicate grinding characteristics for flour production, had an r^2 of 0.98, bushel weight, an r^2 of 0.80, and TKW an r^2 of 0.99 (Garnsworthy et al. 2000). The initial hardness of the grain affects the particle size distribution after grinding and it has been proposed that different calibrations must be developed for wheats with differing hardness (Williams 1979).

The TriQ (BoMill AB; Lund, Sweden) is an NIT instrument that rapidly sorts individual kernels based on chemical and physical attributes (Tønning et al. 2009). The use of NIT spectroscopy in commercial situations requires that the technology be fast, within 5 to 30 milliseconds (ms) for each recording (Berghmans et al. 2012), while maintaining reasonable accuracy and precision. The TriQ contains a rotating steel drum with laser etched grooves. The TriQ steel drum contains 88 laser etched grooves in a row specific for the grain (Lofqvist and Larsson 2015). The machine uses 18 detectors which work with a computer system to determine which fraction each kernel will enter (Lofqvist and Nielsen 2007; Lofqvist and Larsson 2015). As the drum rotates, each kernel rotates in the drum and enters a pocket. Each kernel is then detected by three NIT detectors that transmit light between 1100 nm and 1700 nm, and the resulting data are analyzed using a multivariate system pretreated by an unsupervised multiplicative scatter correction to reduce spectral scatter. The drum allows each kernel to randomly position itself within its own pocket due to the centrifugal force of the drum, which rotates at 60 rpm during machine operation (Lofqvist and Nielsen 2007; Kautzman et al. 2015b; Kautzman et al. 2017). This allows the prediction of CP using a built-in algorithm (BoMill AB, 2008; Patent 7417203). Multiple detectors ensure enough regions within a kernel are measured. The TriQ compromises on accuracy to improve precision so that consistent readings are observed over the batch and a higher sorting capacity is achieved.

Since the TriQ uses transmittance instead of reflectance technology, it is not affected by variability in kernel thickness, but it is dependent on a homogeneous surface texture (Schaare and Fraser 2000). However, reflectance and transmittance are prone to errors due to instrument deviation over time and calibration continually needs to be upgraded when new outliers arrive. The calibrations associated with the TriQ were developed by the manufacturer and are based on narrow wavelength bands, which improves the ability of the machine to scan multiple regions of a kernel at a higher rate. Löfqvist and Nielsen (2007) indicated that NIRS or NITS would be

characterized by multivariate and intensity variation between kernels, allowing more robust calibrations to be developed. Tønning et al. (2009) identified that the TriQ could sort samples using calibrations based on chemical analysis as well as using the NIT spectra alone, with the principal component analysis incorporated into the software.

Previous work tested approaches using laboratory scale seed sorting equipment, where individual grain kernels were exposed to a tungsten lamp and light in the desired spectral region was filtered and recorded using a diode array spectrometer (Löfqvist and Nielsen 2007). The TriQ permits faster sorting and offers large-scale sorting capability. This technology is still novel and a limited number of published studies are available. Kautzman et al. (2015b) used the TriQ to sort wheat based on CP content which was found to be negatively correlated to the deoxynivalenol (DON) content of each kernel (Kautzman et al. 2015b).

2.3 Processing of Grain

2.3.1 Grinding

Feed production can vary in complexity, but the overall goal of a feeding program is to minimize cost to maximize profit (Amerah et al. 2011). Growth and feed intake can be affected by the physical form of the feed, complexity of the diet, major ingredient composition, hardness of the grain, methods used to reduce particle size, and the quality of the final feed (pellet quality and particle size distribution) (Amerah et al. 2011).

Feed production starts when grains received are ground to reduce particle size and increase surface area. Grinding enhances nutrient digestibility and, consequently, improves feed efficiency and animal performance (Abdollahi et al. 2013). While several different grinding approaches are used in a feed mill, hammer milling and roller milling are the most common. In a hammer mill, material that is flowing at a low velocity is impacted by a set of hammers rotating at a higher velocity, while roller mills operate by compressing the materials between rotating pairs of rollers (Abdollahi et al. 2013). Roller mills create a more uniform product (Wondra et al. 1995). The grain, or mixed feed, may be pelleted after grinding. Pelleting incorporates heat and moisture into the ground grain, which is then forced through a die with pores set to a specific

length and diameter (Amerah et al. 2011). Grinding and pelleting have been reviewed extensively, but previous work has been limited to examining batch averages. Primarily, this was due to the lack of technology available to sort grains. If grain can be segregated based on chemical or physical characteristics, the resulting fractions may yield results that are unique to the characteristics of the grains.

The processing index (PI) is used to determine grinding characteristics. Geometric mean diameter provides an accurate measurement of particle size. The DGM is more variable with hammer milling (Amerah et al. 2007b; Amerah et al. 2007a). Differences in particle size are due to the method of grinding, type of grain and pre-grinding treatment; the final product can differ in particle size index, PI and degree of fines. The type of grain will affect the processed particle size, even when the same hammer mill with the same screen size is used (Amerah et al. 2007a). Geometric mean diameter is affected by the type of grain and other ingredients in the diet, type of grinder (hammer mill or roller mill) and post grinding procedures (pelleting or extrusion) (Lentle et al. 2006). Increasing the fines results in an increase in surface area and volume allowing easier access for digestive enzymes and a consequent increase in digestive efficiency (Amerah et al. 2007a).

The efficiency of grinding and DGM are impacted by the hardness of the grain which is due to the resistance of the anatomical parts and the bulkiness of the endosperm (Amerah et al. 2007a). Amerah et al (2007a) indicated that the harder endosperm of hard wheat produced larger particles with more irregular shapes, while soft wheat endosperm produced smaller size particles with more regular shapes. Wheat hardness was negatively correlated to grinding time and the DGM (Pomeranz et al. 1985). Lentle et al. (2006) defined hardness due to the proportion of fines, with higher hardness producing fewer fine particles. Grinding efficiency is affected by hardness, tempering conditions, energy consumption during grinding and the final properties of the ground materials.

2.3.2 Pelleting

Pelleting is a process whereby the feed is exposed to shear force, high temperature (contributed by the shear force and steam incorporated into the conditioner), compression and

friction (Lahaye et al. 2008). Pelleting is the most common hydrothermal treatment and consumes a greater proportion of energy on a per tonne basis than grinding. Pelleting decreases the segregation of ingredients, increases bulk density, and improves handling ease and palatability. The exposure of ingredients to steam within the conditioner causes thermal modification of starch and protein (Chae and Han 1998). Pelleting at high temperatures results in increased starch gelatinization and decreased solubility (Lahaye et al. 2008).

Pelleting can cause excessive heat, resulting in protein damage (Ean et al. 1980). Heat treatment in the presence of moisture results in Maillard reactions, which are a cascade of reactions whereby reducing sugars are heated in the presence of free amino groups, especially lysine (Deng et al. 2005; Odjo et al. 2012). Maillard reactions result in a loss of nutritional and functional properties of protein molecules, may alter flavour or colour (Odjo et al. 2012) and may produce anti-nutritional or toxic compounds (Deng et al. 2005). Maillard reaction products are resistant to enzyme digestion (Deng et al. 2005) reducing lysine availability (Zhang et al. 2008). In the feed industry, Maillard reaction products result from heat applied through steam or pressure. Regulating these may result in the reduction of Maillard reaction products and thus increase nutrient availability. Conversely, Maillard reaction products may be used in the pet food industry to limit digestibility to reduce the potential for obesity (Tran et al. 2008; van Rooijen et al. 2013; van Rooijen et al. 2014).

2.4 Conclusions

The large variability in chemical and physical traits of wheat or barley between batches and between kernels is evident. Some of these differences are due to the type of grain (variety and type of cereal), environmental conditions, and management practices used by the producer. There is limited work on the effect of this variability on feed value and how processing should be adjusted to optimize the nutrient profile of grains.

The reason for the lack of studies on variability between individual kernels has been due to the lack of equipment that allows for the separation of individual kernels into separate fractions based on specific traits. The use of NIRS technology shows promise as it can non-destructively separate individual kernels. This technology will continue to improve and become

more effective with more research and more robust calibrations allowing a determination of its potential for the feed industry.

2.5 Hypothesis

It was hypothesized that using NIT to sort wheat or barley based on predicted CP content would produce fractions that respond differently to processing, which would ultimately impact DM and starch digestibility

2.6 Objectives

The objectives were:

1. To assess the impact of fractionation of individual wheat kernels, using NIT to predict CP content, on the physical characteristics of kernels within each fraction;
2. To determine the impact of sorting wheat or barley, using NIT to predict CP content, on nutrient digestibility in ruminants;
3. To establish if fractions produced by separation, based on predicted CP content using NIT, would contribute to different particle size, and digestibilities.

3 THE EFFECT OF SORTING GRAIN USING CRUDE PROTEIN PREDICTED FROM NEAR INFRARED TRANSMITTANCE SPECTROSCOPY ON THE PHYSICAL TRAITS OF INDIVIDUAL KERNELS AND THEIR RESPECTIVE BULK DENSITY

3.1 Abstract

Advancements in near infrared transmittance spectroscopy (NITS) have allowed the development of commercial technology that can sort individual kernels based on predicted crude protein (CP) content. The objective of this experiment was to determine if fractions obtained by an instrument calibrated to separate individual wheat kernels based on predicted CP content will have different physical characteristics which could impact processing. Six distinct sources of feed grade wheat were collected from Canadian producers and fractionated using a BoMill TriQ NIT seed sorter. Ten fractions were produced from each of the six sources of wheat and the following parameters were measured: major, minor, and intermediate diameters, major and intermediate perimeters and areas, DGM, sphericity, colour and thousand kernel weight (TKW). Diameters, perimeters, DGM, sphericity and TKW differed ($P < 0.05$) between fractions. HunterLab indicated that red/green, yellow/blue, total colour difference, chroma difference and hue angle did not differ between fractions ($P > 0.05$) but lightness did ($P < 0.10$). Fractions obtained using NIT calibrated for predicted CP content did not differ in physical characteristics but did differ in lightness. This variation would be attributed to a relationship between predicted CP content and lightness of the kernel.

Keywords: Fractionation, Near infrared transmittance spectroscopy, grain variability, whe

3.2 Introduction

Variation in the physical and chemical characteristics of wheat and barley has been extensively investigated (Kong et al. 1995; Cai et al. 2013; Shewry et al. 2013). This variation could be due to differences in growing conditions, geographical location, and grain variety (Terman et al. 1969). For example, the average crude protein (CP) content of individual kernels within a sample of wheat or barley ranged from 6 to 20% (Wilkins et al. 1993) or 8 to 15%, respectively (Shewry 2007).

Kernel size, shape (Marshall et al. 1986b; Marshall et al. 1992; Gürsoy and Güzel 2010) and weight (Ohm et al. 1998) are viewed as important physical properties for grain evaluation. Kernel shape varies between species but is less variable within a variety (Harper et al. 1970; Marshall et al. 1984b). Within a plant, the second floret kernel is larger and heavier than the other florets, and the apical spikelets have lower kernel weight and CP content than other kernels in the spikelet (Harper et al. 1970; Marshall et al. 1984b). Technological advancements have improved the ability to measure individual kernel dimensions (Symons and Fulcher 1988a; Symons and Fulcher 1988b; Delwiche and Kim 2000) including the major diameter (L), measured as the longest horizontal cross section through a centre point when observing the crease, and the intermediate diameter (D), which is the shortest cross section through a centre point (Mohsenin 1986). The minor diameter (H) is the minimum diameter when the image is photographed 90° to the L diameter (Mohsenin 1986). Diameters measured are shown in Figure 3.1. Major area and L perimeter are determined by examining the major projected area, whereas the D area and D perimeter are determined by measuring the smallest projected area (Mohsenin 1986).

Near infrared transmittance spectroscopy (NITS) is a spectroscopic approach that uses an electromagnetic spectrum of 780 to 2500 nm to predict chemical composition using calibrations based on wet chemistry (McClure 2003). This technique is non-destructive and provides a rapid assessment of quantitative and qualitative characteristics The BoMill TriQ (TriQ; BoMill AB, Sweden) is a single kernel NITS unit that contains cells that house individual kernels, which are then scanned multiple times with multiple detectors to predict the CP content based on a

transmittance spectrum.

The accuracy of NITS is limited by the accuracy of the wet chemistry methods used when calibrations were developed (Jancewicz et al. 2016). Near infrared transmittance spectroscopy has been used to predict the chemical composition of numerous feed and food samples in the agricultural industry (Corson et al. 1999; McClure 2003; Maslovarić et al. 2011). Near infrared transmittance spectroscopy is affected by differences in particle size and shape due to changes in light scatter (Isaksson and Næs 1988; Pedersen et al. 2002; Rinnan et al. 2009). Various algorithms can be used to correct for these effects during calibration development or when analyzing new samples of interest (Cen and He 2007; Rinnan et al. 2009; Rinnan 2014); however, the sensitivity of NITS to particle size may still result in changes in spectra from different kernel dimensions (Cen and He 2007). Kernel size is correlated to chemical traits such as water hydration capacity (Morgan et al. 2000), chemical composition and hardness. Kernels differing in size and shape require processing adjustments (Gaines et al. 1997; Dziki and Laskowski 2004; Ahmad et al. 2010). If relationships between these traits and kernel dimensions can be identified, it may be possible for NITS to predict these differences by observing the changes in transmittance data alone.

The objective of this study was to assess the physical differences between fractions of wheat kernels separated using NIT based on predicted CP content. It was hypothesized that each fraction would possess different physical traits that could be linked to the predicted CP content.

3.3 Materials and Methods

3.3.1 Sources of Grain

Six independent sources of feed grade wheat (~6 tonnes per source) were purchased from different grain producers in western Canada. Four sources had been downgraded due to Fusarium damage and were classified as either Canada Western Red Spring (CWRS, $n = 2$) or Canada Western Soft White Spring (CWSWS; $n = 2$). Two other CWRS wheats had been downgraded to feed because they did not meet criteria for the lowest food grade defined by the Canadian Grain Commission (https://www.grainscanada.gc.ca/index-eng.htm, 2015).

3.3.2 Single Kernel Near Infrared Transmittance Spectroscopy (NITS) Sorting

The TriQ consists of a drum that rotates at 60 rpm, with 256 rows of 88 laser-etched singulator pockets to position individual kernels. Each drum pocket is sized to the specific grain. Prior to sorting, the TriQ was configured for either wheat or barley. The TriQ incorporates a detector that detects transmittance of individual kernels 18 times. The type of energy source and detectors used by the TriQ is unknown as it is a proprietary. Initially, each source of grain (500 kg representative sample) was passed through the machine to produce ten calibration fractions as described by Kautzman et al. (2015b). Fractions one and ten contained kernels identified by the system as outliers. Outliers were defined as kernels too large to fit into the pocket within the drum, kernels that were too small, when two or more kernels were trapped within a pocket or when kernels did not enter the pocket in the correct orientation. These ten calibration fractions and the original unsorted grain were sampled by taking two samples of 500 grams from each fraction. These samples were stored at -20°C until analyzed.

3.3.2.1 HunterLab Colourimeter

The colour of each duplicate sample was assessed using a HunterLab colourimeter (ColourFlex EZ; HunterLab Inc., Reston, VA, USA) to determine the degree of white to black (L*), red to green (a*) and yellow to blue (b*) as described by Odjo et al. (2011). Colour intensity (ΔE; Equation 3.1), chroma (ΔC; Equation 3.2) and hue angle (HU; Equation 3.3) were calculated as described by Wrolstad and Smith (2003).

$$\Delta E = ((L^*)^2 + (a^*)^2 + (b^*)^2)^{0.5} \quad (3.1)$$

$$\Delta C = ((a^*)^2 + (b^*)^2)^{0.5} \quad (3.2)$$

$$HU = \tan^{-1}\frac{a^*}{b^*} \quad (3.3)$$

All samples were analyzed for colour in a single session to minimize the potential effect of storage on colour change.

3.3.2.1.1 Microscopic imaging of individual kernels

From each fraction produced from each of the six sources of wheat, a minimum of one

hundred kernels were imaged as determined by the power analysis. Individual kernels were fixed on to a microscopic slide allowing the ventral side to be photographed using a dissecting microscope. The kernels were then rotated 90° to be photographed again under a dissecting microscope. These two photographs were used to measure the L and D; L perimeter (calculated as the perimeter of the kernel when measuring L); and L area (calculated as the area of the kernel when the crease is up), H, D perimeter, and D area as described by Mohsenin (1986). Perimeter and area were automatically calculated by measuring pixels using the software AxioVision (Zeiss, Germany 2015). Additionally, the major and intermediate roundness (Equations 3.4 and 3.5), DGM (Equation 3.6), and sphericity (ϕ, Equation 3.7) were calculated using data obtained from the dorsal and ventral sides of individual kernels.

$$\text{Major Roundness} = \frac{(\text{Major Perimeter})^2}{((4\pi)\times\text{Area})} \tag{3.4}$$

$$\text{Intermediate Roundness} = \frac{(\text{Intermediate Perimeter}\times\text{Major Perimeter})^2}{((4\pi)\times\text{Area})} \tag{3.5}$$

$$\text{Geometric mean diameter} = \sqrt[3]{(\text{Minor diameter} \times \text{Intermediate diameter} \times \text{Major Perimeter})} \tag{3.6}$$

$$\text{Sphericity } (\psi) = \frac{\sqrt[3]{(\text{Minor diameter}\times\text{Intermediate diameter}\times\text{Major Perimeter})}}{\text{Major diameter}} \tag{3.7}$$

Standard deviation for each measurement was calculated to determine the variation within a fraction.

3.3.2.1.2 Thousand Kernel Weight (TKW)

Thousand kernel weights (TKW, mg) were determined in triplicate using an ESC-1 seed counter (model ESC120006; Agriculex Inc., Guelph, ON, CAN). The seed counter was calibrated by adjusting its sensitivity until 100 kernels were accurately measured to a ± 1. After 1000 kernels were counted, they were weighed in mg to a ± 0.001.

3.3.2.1.3 Statistical Analysis

Normality of data was assessed using Shapiro-Wilk W Statistic (Proc Univariate, SAS v9.4; SAS Institute, Cary, NC) where non-significance indicates normality. If data were not normal, then data were transformed using the lambda procedure. The experiment was a completely random design (CRD) analyzed using a mixed model (Equation 3.8; Proc Mixed,

SAS 9.4; SAS Institute, Cary, NC) to evaluate differences between fractions as well as differences between types of wheat (CWRS and CWSWS) for kernel morphology and colour. Six sources of wheat and samples from each of the ten calibration fractions from each source were evaluated. Data were initially analyzed using a random complete block design (Equation 3.9) by type of wheat and, if no difference was found between blocks, CRD was performed as shown in Equation 3.8. In addition, standard deviations (SD) for each fraction of each measurement was calculated and these were compared to determine variability within a fraction using a CRD and analyzed as a mixed model (Proc Mixed, SAS v 9.4; SAS Institute, Cary, NC) to evaluate whether variation within a fraction differed among fractions for kernel morphology or colour (Equation 3.8). The models used were:

$$Y = \mu + \alpha_i + \varepsilon_i \quad (3.8)$$

$$Y = \mu + p_i + \alpha_j + \varepsilon_{ijk} \quad (3.9)$$

Where μ was the overall mean, α was the main effect of fractionation, p was the block and type of grain and ε_i was the residual error. Significance was declared when $P < 0.10$ and a tendency was discussed when $0.10 < P < 0.15$.

3.4 Results

All variables, except a^*, differed between wheat types (Table 3.1; $P > 0.05$) with CWSWS having higher values than CWRS. No interactions were observed between type of wheat and fraction for colour variables (Table 3.1; $P > 0.05$*)*. The a*, b*, ΔE and ΔC measurements were similar among fractions ($P > 0.10$) but L* was different between fractions ($P < 0.10$). The L* of fractions 8, 9 and 10 was greater than that of fractions 1 through 7. Hue angle differed between fractions ($P = 0.03$) with fractions 8 to 10 (2.66, 2.63 and 2.67, respectively) different from fraction 1 (2.95). There was a tendency for light intensity to be highest for fractions 1 to 3 and lowest for fractions 8 to 10 (Table 3.1; $P = 0.15$*)*. There was no difference among fractions for TKW (Table 3.1, $P = 1.00$*)* or colour (Table 3.1; $P > 0.10$) except L* where a difference was observed between fractions (Table 3.1; $P = 0.10$). Thousand kernel weights and all colour variables except a* differed between CWSWS and CWRS (Table 3.1; $P < 0.10$).

3.4.1 Kernel Dimensions

3.4.1.1 Average Kernel Dimensions

No interaction was observed between type of wheat (CWSWS versus CWRS) and fraction for dimensional measurements. The L, D and H diameter, D area, L perimeter and D perimeter, L roundness, DGM and sphericity (Table 3.2: $P < 0.10$) were different between types of wheat; L area, D roundness and mass (Table 3.2: $P > 0.10$) did not differ between types of wheat. No differences in dimensional measurements were observed between fractions (Table 3.2: $P > 0.10$).

3.4.1.2 Variation in Individual Kernel Morphological Characteristics

Variation among fractions for each measurement is described by the SD (Table 3.3). For all variables examined, no interaction was observed between fraction and type of wheat ($P > 0.10$). Significant variation within fractions for the L, D and H diameter, L area, D area, L perimeter, DGM, sphericity and mass measurement were observed with fraction 1 usually different from fraction 9 ($P < 0.10$). However, the variation within fractions for the D perimeter, L round and D round were similar ($P = 0.67$, 0.89 and 0.98, respectively).

3.5 Discussion

Grain is currently cleaned and sorted using physical characteristics such as size, colour and density, primarily to increase the uniformity prior to further processing (Graybosch et al. 1995). When wheat was sorted into fractions using an NIT instrument (TriQ) calibrated to predict CP content; each fraction produced flours of different baking quality (Tønning et al. 2009). Potential changes in morphology of individual kernels were not examined in this study (Tønning et al. 2009). The TriQ is reported by the manufacturer to fractionate grain based on predicted CP content; however, data validating the ability to sort grain is limited. Furthermore, there is no information examining the effect of fractionation on individual kernel physical characteristics, which can be an important factor when considering further processing.

Thousand kernel weight (TKW) provides an objective measurement that evaluates a sample but does not explain the variation within the sample (Armstrong et al. 2002; Whan et al.

2014). As shown in Table 3.1, the TKW was similar among fractions, indicating that the bulk density of the fractions was similar. Previous work has indicated that there is a strong correlation, but not a linear relationship, between TKW and kernel dimensions (KD), with kernel length and kernel width having a greater influence than KD ratio (Cui et al. 2011). Thousand kernel weight is an important parameter for grain millers (Armstrong et al. 2002) and is used by feed producers as a measurement of quality. High TKW kernels are plumper, have higher endosperm content (Armstrong et al. 2002) and higher flour yield than lower TKW kernels (Marshall et al. 1984a; Marshall et al. 1986a). This indicates that a high TKW is likely to have a higher level of starch and lower CP content. It can be hypothesized that differences in size between CWSWS and CWRS are due to differences in starch and/or CP content.

A second characteristic affecting the value of wheat is colour. The ten fractions produced by the TriQ differed in L* and HU, but in none of the other colour parameters. The darkness of the kernel cannot affect NIT as electromagnetic radiation enters a sample with a negligible amount of scatter due to the size of the kernel (Huang et al. 2008). Previous work has found that wheat kernels vary in colour from light yellow to red brown, influenced by the amount of red pigmentation in the seed coat (Wang et al. 1999) and bleaching (Venora *et al.* 2009). Differences in wheat colour can be attributed to several factors such as genetics (Baker 1981), solar radiation and variety (Lukow et al. 2012), and water stress (Ozturk and Aydin 2004). It has been shown that red wheat colour is dominant to white wheat colour (Corpuz et al. 1983). Future work will be necessary to identify if the colour variation observed affected the chemical traits within each fraction. Previous work has reported that sorting single samples of wheat based on colour impacted milling, baking and sensory properties (Wang et al. 1999; Pasikatan and Dowell 2003). The sources of grain used in this study were feed grade and thus how they may change in processing, chemical traits and palatability needs to be investigated further. Colour could be different due to differences in chemical traits between the fractions. Previously, Kautzman et al. (2015b) observed significant differences in CP content between the ten fractions produced by the TriQ. Kautzman et al. (2015b) sorted fractions to remove fusarium damaged kernels which were found to be correlated to CP.

In the food industry, fractions of wheat differing in colour can result in different prices

(Wang et al. 1999). Grain inspection is performed visually by kernel size, shape and colour (Pasikatan and Dowell 2003), and thus fractions differing in colour could be graded differently, creating a more uniform product. For low grade wheat this could result in certain fractions within this grade being upgraded to a higher grade or being priced differently due to perception.

Commercial colour sorters using optical imaging sort wheat at a rate as high as 1200 kg h^{-1} (Pearson et al. 2008), but currently neither NITS nor NIRS is used commercially for colour sorting. The colour sorter has been used to remove ergot infested kernels from grain as well as to separate red kernels from white kernels. The disadvantage of a colour sorter is that it removes kernels that could be used by the processor (Pearson et al. 2008). Colour sorting technology has become the most successful technology in separating grain based on colour. Further work will be required to identify the return to investment for such technology within Western Canada and if there is value in its use in the feed industry.

The variability within a fraction explained by SD was highest for fractions 1 and 10 when assessing the dimensions of a kernel. Fractions 1 and 10 contained outliers as well as the lowest and highest 10% predicted CP content, respectively. Outliers will be kernels that are either too small, too large, or have predicted CP content that are not within the calibration curve. These outlier kernels within the fraction will explain the higher SD in these fractions.

Dziki and Laskowski (2004) separated *Triticum* kernels into three classes based on kernel size (small: 2.0 to 2.5 mm, medium: 2.7 to 2.9 mm, large: 3.1 to 3.5 mm). The larger wheat kernels were softer, had lower ash content, and higher bulk density than the smaller kernels (Dziki and Laskowski 2004). Sorting kernels using the TriQ did not have a significant effect on kernel size. Geometric properties such as sphericity and DGM have an influence on density which can affect the design of equipment used (Gürsoy and Güzel 2010). The TriQ uses a drum with specific pores for each type of grain. The limited variability in drum pores may limit the variability in kernels to be sorted. In this study, no variability in dimensions was observed between fractions 2 to 9. Previous work conducted by O'Neil et al. (1999) showed that NIRS was sensitive to particle size and thus the TriQ must have reduced this sensitivity by limiting the size of the kernel being detected by the detector.

Sorting based on predicted chemical composition of individual kernels can impact the use and value of the grain. Environmental conditions such as length of season, nutrient availability, and management practices can affect the amount of starch and protein incorporated (Edwards 2010). These changes are likely to result in differences in physical traits such as kernel dimensions or colour. Crop maturity and growing conditions affect deposition of starch and protein (Jenner et al. 1991). These could influence the value of the crop to the animal, but further work will be required to determine how each fraction produced by the NIT differed in its chemical properties and how it could affect digestibility and nutrient value and if there is any economic gain in the use of such a tool.

3.6 Conclusions

The TriQ, an NITS, produced ten fractions that differed in L* and HU, but not their physical characteristics. Colour traits of fractions were different, indicating a potential chemical difference. Future work will be necessary to identify the different methods of sorting on an individual kernel basis (sorting using the spectroscopy approach versus sorting based on physical traits) and if different methods of processing will be required for each fraction. In addition, a more extensive understanding of how the chemical composition of each fraction differs from the unsorted sample is required to fully understand the value of sorting on an individual kernel basis. Sorting using an NIT instrument should be preceded by separation based on size to improve the uniformity of the kernels to ensure an appropriate drum is used. This has the potential to reduce the number of outliers and improve the accuracy and precision of the NIT.

3.7 Next stage

The results in this chapter indicated that kernel physical characteristics did not vary between fractions produced when grain was sorted using single kernel NITS. This indicates that either the predicted CP content between fractions did not result in differences in the size of the kernels or, the fractions did not differ chemically. The next stage of this project was to determine the impact of sorting wheat or barley, using a single kernel NITS based on predicted CP content, on nutrient digestibility in ruminants

3.8 Acknowledgements

This study was completed at the Canadian Feed Research Centre (CFRC, North Battleford, Canada), the Department of Pathology at the Western College of Veterinary Medicine, (University of Saskatchewan, Saskatoon, Canada), and the Department of Animal and Poultry Science. The author thanks Scott Bishop at the CFRC for his assistance in producing the fractions from the six sources used in this study. The author would also like to thank Chris Stuart and Ian Shirley for their assistance at the Department of Pathology in learning how to utilize the software used in measuring kernel dimensions. In addition, the author would like to thank Dr. Tom Scott for his input. This study was made possible by a grant from the Alberta Crop Industry Development Fund.

3.9 Conflict of interest

None

Figure 3.1. Image of two kernels with the longest dimension represented as the major diameter (L), the shortest diameter is referred to as the minor diameter (H) and the intermediate diameter (D) representing the width of the kernel when measuring the major diameter.

Table 3.1. Mean colour characteristics (HunterLab L* a* b), colour intensity (ΔE), chroma (ΔC) hue angle (HU) and thousand-kernel weight (TKW) for six[Z] independent samples of two types of wheat (Canadian Western Red Spring (CWRS) and Canadian Western Soft White Spring (CWSWS)) sorted using the BoMill TriQ single kernel near infrared transmittance spectra seed sorter to produce 10 fractions based on predicted CP content.

	Type of wheat (TW)				Fraction											
Fraction	CWSWS	CWRS	SEM[Y]	*P-value*	1	2	3	4	5	6	7	8	9	10	SEM	*P-value*
L*	53.09	51.91	0.460	*0.03*	54.50a	53.81a	53.39a	52.62a	52.70a	52.05a	51.63a	51.48b	51.41b	51.42b	0.841	*0.10*
a*	8.48	8.47	0.074	*0.91*	8.21	8.44	8.44	8.54	8.4	8.54	8.46	8.64	8.57	8.47	0.135	*0.67*
b*	26.19	22.42	0.301	*0.01*	25.11	24.9	24.68	24.5	24.31	24.35	23.98	24.00	23.66	23.59	0.549	*0.58*
ΔE	59.81	57.18	0.519	*0.01*	60.59	59.92	59.44	58.7	58.67	58.12	57.57	57.48	57.26	57.22	0.948	*0.15*
ΔC	27.53	23.91	0.295	*0.01*	26.43	26.3	26.09	25.95	25.73	25.82	25.43	25.52	25.17	25.08	0.589	*0.70*
HU	2.983	2.524	0.034	*0.01*	2.95a	2.83ab	2.81ab	2.75ab	2.78ab	2.74ab	2.72ab	2.66b	2.63b	2.67b	0.062	*0.03*
TKW (mg)	36.18	28.63	1.784	*0.01*	31.53	32	32.8	32.52	32.92	32.7	33.01	32.89	32.30	31.33	3.257	*1.00*

A minimum of 100 kernels was measured from each sample.

All interactions were $P = 1.00$ except a* which had a $P = 0.63$.

[Z]The six independent samples of wheat were comprised of four sources of CWRS and CWSWS downgraded due to Fusarium damage and two other CWRS samples downgraded due to midge damage and other factors.

[Y]SEM Standard error of the mean.

Table 3.2. Mean major diameter (L), intermediate diameter (D), minor diameter (H), major area (L area), intermediate area (D area), major perimeter (L-perimeter), intermediate perimeter (D-perimeter), sphericity, DGM and mass of individual wheat kernels of six[Z] independent samples of two types of wheat (Canadian Western Red Spring (CWRS) and Canadian Western Soft White Spring(CWSWS)) for the 10 fractions based on predicted CP content obtained using the BoMill TriQ single kernel near infrared transmittance spectra.

	Type of wheat				Fraction											
	CWSWS	CWRS	*SEM*[Y]	*P value*	1	2	3	4	5	6	7	8	9	10	*SEM*	*P value*
L diameter (mm)	5.52	5.73	0.037	*0.01*	5.55	5.63	5.65	5.65	5.63	5.62	5.66	5.64	5.63	5.56	*0.072*	*0.98*
D diameter (mm)	3.16	3.05	0.024	*0.01*	3.01	3.10	3.13	3.13	3.12	3.15	3.13	3.12	3.12	3.07	*0.046*	*0.67*
H (mm)	2.70	2.82	0.018	*0.01*	2.69	2.77	2.79	2.79	2.77	2.75	2.80	2.76	2.77	1.72	*0.036*	*0.61*
L area	13.61	13.57	0.188	*0.84*	13.03	13.63	13.79	13.79	13.68	13.64	13.78	13.67	13.67	13.27	*0.364*	*0.89*
D area	11.32	12.19	0.134	*0.01*	11.40	11.91	11.96	11.97	11.85	11.72	11.91	11.62	11.79	11.42	*0.260*	*0.72*
L round	1.20	1.25	0.006	*0.01*	1.24	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	*0.013*	*0.97*
D round	1.34	1.35	0.006	*0.31*	1.35	1.34	1.34	1.34	1.34	1.34	1.35	1.34	1.34	1.35	*0.012*	*0.99*
L perimeter (mm)	14.25	14.52	0.063	*0.02*	14.16	14.39	14.47	14.47	14.40	14.40	14.49	14.43	14.40	14.23	*0.172*	*0.94*
D perimeter (mm)	13.73	14.30	0.082	*0.01*	13.84	14.13	14.13	14.12	14.08	13.98	14.16	13.91	14.02	13.83	*0.159*	*0.78*
Sphericity (%)	65.38	64.02	0.211	*0.01*	64.00	64.67	64.91	64.93	64.82	64.70	64.84	64.70	64.82	64.61	*0.408*	*0.91*
DGM (mm)	3.61	3.66	0.023	*0.06*	3.55	3.64	3.66	3.66	3.64	3.64	3.67	3.64	3.65	3.59	*0.044*	*0.70*
Mass (mg)	33.98	34.29	0.753	*0.73*	30.68	34.11	34.44	35.75	34.23	34.14	35.06	34.69	34.96	33.29	*1.458*	*0.53*

A minimum of 100 kernels was measured from each sample.

All interactions had a P value greater than 0.10.

[Z]The six independent samples of wheat were comprised of four sources of CWRS and CWSWS downgraded due to Fusarium damage and two other CWRS samples downgraded due to midge damage and other factors.

[Y]SEM: Standard error of the mean.

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Table 3.3. Standard deviation of microscopic and mass measurements of individual wheat kernels for the 10 fractions obtained based on predicted CP content using the BoMill TriQ single kernel near infrared transmittance spectra for six[Z] independent samples of two types of wheat (Canadian Western Red Spring (CWRS) and Canadian Western Soft White Spring (CWSWS) sorted using the BoMill TriQ single kernel near infrared transmittance spectra seed sorter to produce 10 fractions based on predicted CP content.

	Type of wheat				Fraction											
	CWSWS	CWRS	*SEM*[Y]	*P value*	1	2	3	4	5	6	7	8	9	10	*SEM*	*P value*
L[X] diameter	0.358	0.393	*0.013*	*0.01*	0.421a	0.390ab	0.400a	0.377ab	0.375ab	0.364ab	0.362ab	0.348ab	0.331b	0.386ab	*0.025*	*0.01*
D[W] diameter	0.327	0.293	*0.007*	*0.01*	0.359a	0.331ab	0.315ab	0.319ab	0.313ab	0.303ab	0.304ab	0.292b	0.269b	0.294b	*0.014*	*0.01*
H[V] diameter	0.254	0.259	*0.007*	*0.44*	0.300a	0.259ab	0.256ab	0.257ab	0.250ab	0.242b	0.276ab	0.242b	0.238b	0.248b	*0.011*	*0.01*
L area (mm^2)	2.009	1.889	*0.050*	*0.06*	2.210a	2.091ab	2.006ab	2.006ab	1.979ab	1.905ab	1.941ab	1.797ab	1.713b	1.817ab	*0.097*	*0.03*
D area (mm^2)	1.603	1.673	*0.049*	*0.05*	1.871	1.692	1.677ab	1.651ab	1.588ab	1.584ab	1.540ab	1.773ab	1.455b	1.548ab	*0.094*	*0.08*
L[X] perimeter	1.015	1.020	*0.040*	*0.72*	1.150a	1.039ab	1.044ab	1.069ab	1.010ab	1.005ab	1.008ab	0.996ab	0.868b	0.988ab	*0.076*	*0.09*
D[W] perimeter	1.029	1.039	*0.060*	*0.07*	1.090	1.042	1.019	0.944	0.958	0.975	1.138	1.271	0.881	1.021	*0.115*	*0.67*
L[X] round	0.073	0.087	*0.008*	*0.36*	0.106	0.072	0.074	0.079	0.073	0.078	0.087	0.084	0.063	0.087	*0.016*	*0.86*
D round	0.096	0.103	*0.010*	*0.11*	0.090	0.098	0.101	0.083	0.084	0.095	0.141	0.099	0.087	0.116	*0.021*	*0.98*
DGM[U] (mm)	0.267	0.255	*0.006*	*0.14*	0.313a	0.279ab	0.271ab	0.265ab	0.262ab	0.252b	0.258ab	0.235b	0.228b	0.247b	*0.012*	*0.01*
Sphericity (%)	3.214	3.230	*0.074*	*0.86*	3.691a	3.257ab	3.309ab	3.232ab	3.016b	3.028ab	3.252ab	3.111ab	2.999b	3.326ab	*0.143*	*0.05*
Mass (mg)	0.008	0.007	*0.001*	*0.02*	0.009a	0.008ab	0.007bc	0.008abc	0.007bc	0.007bc	0.007abc	0.007c	0.006c	0.007bc	*0.001*	*0.01*

A minimum of 100 kernels was measured from each sample.
All interactions had a P value greater than 0.10.
[Z]The six independent samples of wheat were comprised of four sources of CWRS and CWSWS downgraded due to Fusarium damage and two other CWRS samples downgraded due to midge damage and other factors.
[Y]SEM: Standard error of the mean.
[X]L: Major (mm).
[W]D: Intermediate (mm).
[V]H: Minor (mm).
[U]DGM: Geometric mean diameter.

4 THE EFFECT OF SORTING INDIVIDUAL KERNELS OF WHEAT OR BARLEY BASED ON PREDICTED CRUDE PROTEIN CONTENT AND THE INTERACTION WITH PARTICLE SIZE REDUCTION ON NUTRIENT DIGESTIBILITY FOR RUMINANTS

4.1 Abstract

Advancements in near infrared transmittance spectroscopy (NITS) have allowed the development of technology that can sort individual kernels based on predicted crude protein (CP) content. The objective of this experiment was to investigate if the use of an NITS to sort grain based on predicted CP content would result in fractions that differed in *in vitro* starch digestibility. Secondly, we wanted to determine if the starch digestibility of these fractions responded differently to grinding. Five independent sources of feed grade wheat and barley were collected from Canadian producers and fractionated using a BoMill TriQ NITS seed sorter. Three fractions were produced for each of the five sources, the original unsorted (UNSORT) grain and 2 fractions [high CP (HCP) vs. low CP (LCP)]. Each fraction (UNSORT, HCP, and LCP) was ground through a hammer mill with either a 0.375-mm or a 0.188-mm screen using a hammer mill or run through a roller mill to produce coarse and fine treatments. The UNSORT was used to adjust the roller mill to produce ground samples with a similar processing index (w/v) relative to the hammer mill. *In vit*ro DMD and total gas production (TGP) were determined after a 12-h incubation. Data were analyzed independently by grain source including the effect of fraction, grinder type, degree of processing, and their interactions. Fractions for wheat did not differ in their CP ($P = 0.87$) concentration but a tendency for barley was observed ($P = 0.08$). Starch content did not differ between fractions for either wheat or barley ($P = 0.97$ and $P = 0.54$, respectively). Grinding properties were not different between fractions ($P > 0.10$). The 12-h TGP (mL) and DMD (%) of barley ground using a hammer mill was greater than when processed using a roller mill ($P < 0.10$; 59.4 ± 2.0 and 41.8 ± 1.0, respectively). Wheat and barley had a TGP and DMD that did not differ between fractions but did differ between type of grinder and severity of processing ($P < 0.01$) with hammer milling having a higher TGP and DMD than roller milling. Additionally, fine grinding for hammer milling had the highest TGP and DMD. A tendency for wheat and barley was observed for DMD with grinder type × severity of processing

× TriQ fraction ($P = 0.09$ and $P = 0.08$). Fractions (LCP and HCP were different than UNSORT) ground under hammer milling with fine grinding producing the highest DMD. The use of NITS to sort wheat or barley based on CP content did not produce chemical or DMD differences.

Keywords: Near infrared transmittance spectroscopy, grain variability, wheat

4.2 Introduction

Grain contributes up to 90% of the total ration of finishing cattle (Schaefer. 2018). Grain entering a feed production facility is not pre-sorted into fractions based on physical or chemical traits due to the lack of technology and/or research indicating benefits of sorting. Therefore, grain entering a feedlot will be processed using the same protocol, regardless of variation in physical or chemical characteristics within each lot (Kong et al. 1995; Cai et al. 2013; Shewry et al. 2013). However, variation in wheat shape or size can result in the processed product having particles with a DGM that has a high standard deviation (Bramble et al. 2002).

Chemical composition also varies between and within samples. The starch content of barley can be as low as 51% to as high as 64% (Holtekjølen et al. 2006). In addition to variations in the amount of starch, the type of starch can also vary (Baik and Ullrich 2008); the amylose content of barley starch can range from 0% to 5% in waxy barley, 20 to 30% in normal barley and up to 45% in high-amylose barley varieties. This variation in amylose content affects digestibility and processing characteristics. Crude protein content in barley and wheat can range from 8 to 15% or 6 to 20%, respectively (Kirkman et al. 1982; Shewry 2007), due to variation in growing conditions, locations, and variety (Terman et al. 1969).

Grain is rarely fed unprocessed, and particle size reduction is typically accomplished using a hammer or roller mill (Owens et al. 1997; Pritchard and Stateler 1997; Beauchemin et al. 2001). The hull or husk, if present, as well as the protein matrix, play critical roles in the hardness of grains, affecting particle size after grinding and form a barrier to other nutrients within the kernel (Anjum and Walker 1991; Dziki 2008). Hammer milling of cereal grains improves the energy value of the feed more than does roller milling because the greater reduction in particle size results in a higher proportions of fines and an increase in area exposed to digestion even when the same DGM is obtained (Fang et al. 1997; Dziki and Laskowski 2006; Budacan 2012). However, fine particles (particles less than 1 mm in diameter) cause digestive upsets and a reduction in animal performance in ruminants (Owens et al. 1997; Beauchemin et al. 2001; Krause and Oetzel 2006). Previous work examining the effects of grinding on animal performance has been limited to investigations on bulk samples. Separating grain on a single kernel basis to increase uniformity of kernel size entering a grinder has had limited success

(Ahmad *et al.* 2010) due to several factors, including the inability to sort on a commercial scale and limited research on how this may benefit the feed industry. However, in theory, such separation would result in a lower standard deviation of DGM (S_{gw}) of each fraction, and thus reduction in the fines present within the final sample.

The development of an instrument that sorts individual kernels based on predicted CP content (TriQ; BoMill AB, Sweden) allows separation of individual kernels of grain into fractions without destruction, allowing further processing of the fractions. The objective of this experiment was to determine if the use of near infrared transmittance spectroscopy (NITS) with built in calibration to predict CP content would result in fractions that differ in *in vitro* starch digestibility and whether this difference would be affected by the method and degree of grinding.

4.3 Materials and Methods

4.3.1 Initial Grain Sources

Feed grade wheat and barley were purchased from five independent sources located in different areas. The wheat and barley were tested for mycotoxins (Prairie Diagnostic Services, PDS, University of Saskatchewan, Saskatoon, SK. Canada) to ensure levels were below CFIA limits (CFIA 2017). The grain was cleaned of any foreign materials (e.g.: dirt, rocks, chaff, etc.) using an AS4 model 6048 Air and Seed Cleaner (Flaman, Saskatoon Canada) which removes unwanted materials using vibrating sieves.

4.3.2 Sorting of Grains

The grains were sorted using a commercial single kernel NIT sorter BoMill TriQ (TriQ; BoMill AB, Vintrie, Sweden). Initially, 500 kg from each source was sorted to produce 10 fractions, which were used to determine the normal distribution of the predicted CP content within that source. This step was performed to calibrate the TriQ and allow the identification of the three fractions to be used further. The TriQ was then set to enable the collection of three fractions. The sample with lowest CP content (LCP; 30% of the lowest CP; obtained from the three lowest CP fractions of the 10 fractions), a sample with the highest CP content (HCP; 30% with the highest CP, obtained from the three highest CP fractions of the 10 fractions) and the

medium CP fraction, content plus the outliers. The low CP and high CP fractions and the original unsorted grain were used to evaluate whether sorting or grinding method or severity (coarse vs fine grinding) affected dry matter digestibility (DMD) and starch digestibility differentially, according to predicted CP content.

4.3.3 Grinding

Grinding was performed at the Canadian Feed Research Centre (CFRC: North Battleford, SK) and the College of Engineering at the University of Saskatchewan (Saskatoon, SK). A 10 kg sample of each of the three different fractions that were obtained from the TriQ (initial unsorted grain, low CP and high CP fractions) was ground using either a hammer or a roller mill set to achieve either a course or a fine grind (severity of grinding). Sorted fractions were ground using a hammer mill with a 0.375-mm or a 0.188-mm screen opening designed to provide a coarse or fine grind, respectively. The processing index (PI) of the unsorted grain was determined for both coarse and fine grinding by dividing the mass of ground grain that filled a standardized one-hectolitre cup by the initial mass of the unground grain. The roller mill settings were adjusted for the unsorted grain sample to achieve a PI within 5% of that obtained using the hammer mill. The sorted fractions were ground through the same roller mill settings as that established for the unground grain. This process was repeated for each source of wheat and barley.

4.3.4 Particle Size Analysis

Particle size of the ground grain for each fraction was determined using the method described in ASAE (2012) with ten sieves (pore size [US sieve size]) of 3.36 mm [6], 2.38 mm [8], 1.68 mm [10], 1.191 mm [14], 0.841 mm [20], 0.594 mm [28], 0.500 mm [35], 0.297 mm [48], 0.212 mm [65], 0.149 mm [100], 0.103 mm [150], 0.074 mm [200], 0.053 mm [270] and pan) and a rotary sieve shaker for a period of 10 minutes (model Rotary Lab Sifter, Hoskin Scientific Vancouver, British Columbia, Canada). The mass remaining on each sieve was weighed and used to calculate: DGM (Equation 4.1); standard deviation of the DGM (S_{gw}; Equation 4.2); surface area covered by the average particle (Equation 4.3); and number of particles (Equation 4.4) using Equations described by ASAE (2012).

$$d_{gm} = \log^{-1}\left[\frac{\sum(W_i \log d_i)}{\sum(W_i)}\right] \quad (4.1)$$

$$S_{gw} = \log^{-1}\left[\frac{\sum(W_i)(\log d_i - \log d_{gw})^2}{\sum(W_i)}\right]^{1/2} \quad (4.2)$$

$$\text{Surface area } (\frac{cm^2}{gram}) = \frac{6}{16} e^{(0.5\times(LN(S_{gw}))^2 - LN(D_{gw}*0.0001))} \quad (4.3)$$

$$\frac{\text{Particles}}{\text{gram}} = \frac{1}{16} * e^{(4.5\times(LN(S_{gw}))^2 - (3\times LN(D_{gw}*0.0001)))} \quad (4.4)$$

where d_i is the diameter of sieve openings of the i^{th} sieve, d_{i+1} is the diameter of the openings in the next larger i^{th} sieve and W_i. is the weight of each fraction on the i^{th} sieve.

4.3.5 *In Vitro* Gas Production Technique

The *in vitro* gas production technique measured gas production to determine DMD from feed incubated in buffered rumen fluid (Rymer et al. 2005). A total of 0.5 g of ground grain from each treatment was weighed into a nitrogen free bag (ANKOM, ANKOM Technology New York., USA) which had been soaked in acetone for 24 h. After 24 h, the Ankom bags were dried overnight at room temperature. Rumen fluid from two cows was sampled from the anterior, caudal, and mid-ventral regions of the rumen 2 h after the morning feed. The cows had been fed 10% barley silage, 67% barley grain, 20% corn distiller's dried grains with solubles and 3% pellets composed of 54.82% barley chop, 9.70% canola meal, 24.26% calcium carbonate, 2.43% molasses, 4.85% salt, 0.97% feedlot premix (36.56% calcium carbonate, 29.77% zinc sulphate, 10.83% copper sulphate, 15.34% manganous sulphate, 5.29% of selenium premix 1%, 80% of 0.15% ethylene diamine dihydro iodine, 1.80% vitamin A, 0.18% vitamin D, 1.94% urea, 0.06% vitamin E and 0.97% canola oil in a total mixed ration.

The pH of the rumen fluid was determined immediately after being strained using a pH meter (VWR, Mississauga, ON). The rumen digesta was strained through a 355 μm pore size polyester screen (model PeCAP®, Sefar America Inc, New York, USA). If the pH was lower than 5.5, the rumen fluid was not used, and another sample was collected one hour later. The rumen fluid was transferred to an insulated container, gassed with O_2-free CO_2 and transported to the on-site laboratory. The rumen fluid was strained through cheesecloth and then 20 mL of

rumen fluid was mixed with 45 mL of pre-warmed medium (Goering and Van Soest 1970). The media contained 5 g L^{-1} of tryptone, 250 mL L^{-1} buffer solution, 250 mL macromineral solution, 0.125 micromineral solution and 1.25 mL of 0.1% resazurin solution. This media was warmed in a water bath set at 39ºC and bubbled with CO_2 through the solution for 45 min. After 45 min 0.313 g L^{-1} of cysteine hydrochloride and 0.313 g of sodium sulphide was added and then bubbled with CO_2 for 15 minutes. The buffer solution was composed of 4 g L^{-1} of ammonium bicarbonate and 35 g L^{-1} of sodium bicarbonate. The macro-mineral solution was composed of 5.7 g L^{-1} of sodium hydrogen phosphate dibasic and 6.0 g L^{-1} of magnesium sulfate $7H_20$. The micromineral solution was composed of 132 g L^{-1} calcium chloride $2H_2O$, 100 g L^{-1} manganese chloride $4H_2O$, 10 g L^{-1} cobalt chloride $6H_2O$ and 80 g L^{-1} ferric chloride $6H_2O$.

The medium plus the rumen fluid collected from the cows was dispensed anaerobically into 100-mL serum bottles fitted with rubber stoppers which were then flushed with CO_2. The vials were sealed with +14-mm butyl rubber stoppers held in place by an aluminum crimp cap. Vials were incubated at 39°C with mixing on a rotary shaker (120 rpm).

Gas production (GP) was measured at 2, 4, 8, and 12 h after the start of the incubation, by inserting a 23-gauge needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, Quebec., Canada) into the vial, with pressure read by the transducer connected to a visual display (Data Track, Christchurch, UK). The gas was released from each vial following each GP measurement. Pressure values were corrected for substrate incubated and the gas produced from the negative controls by subtracting the gas volume produced by the negative control and then dividing that by the weight of the substrate incubated. Negative controls consisting of rumen fluid with media but no substrate were run in duplicates to correct for endogenous gas production. A second standard (run in duplicate; media with substrate but no rumen fluid) was included in each run to calculate the inter-run CV and adjust values among runs if needed.

Pressure was converted to gas volume (Equation 4.5; adapted from Mauricio et al. 1999).

$$\text{Gas volume} = 0.18 + (3.697 \times \text{gas pressure}) + (0.0824 \times \text{gas pressure})^2 \quad (4.5)$$

Following the 12-h incubation period, the ANKOM bags were removed from the vial and placed on ice to stop fermentation. The ANKOM bags (ANKOM Technology Inc) containing the remaining feedstuff were dried and used for DM and starch analysis. Samples of the original substrate were analyzed for DM and starch.

4.3.6 Chemical analysis of five independent sources of grain.

Percent nitrogen was determined for each fraction using the combustion method (AOAC 968.06; AOAC, 2005) using a Leco FP-528 analyzer (St Joseph, MI). This was converted to CP content using a factor of 5.83 (Merrill and Watt 1955). Starch in the initial fractions and the material remaining after digestion was determined using the Megazyme kit (Megazyme International Ireland, Wicklow, Ireland). This protocol is based on enzyme hydrolysis of α-linked glucose monomers and the glucose was determined colorimetrically at 550 nm.

4.4 Experimental Design

To evaluate differences in starch and CP content between each fraction obtained using a TriQ and the initial unsorted fraction, a completely random design was performed. Wheat and barley were analyzed separately. The model represents an *n* of five sources for wheat or barley. The model was:

$$Y = \mu + \alpha_i + \varepsilon_i \quad (4.6)$$

Where μ was the overall mean, α was the main effect of fractionation using the TriQ and ε_i was the residual error.

The experiment for the *in vitro* digestibility was designed as a split plot design and analyzed using a mixed model (Proc Mixed, SAS 9.4: SAS Institute, Cary, NC). The main plot was fraction and the subplots were grinder and severity of grinding. The model contained effects of fractions, grinders, severity of grinding and interactions.

The model used to analyze the three fractions (unsorted, LCP and HCP) from each of the five sources of wheat or barley was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\gamma\beta)_{kj} + (\alpha\gamma)_{ik} + c_{ijk} + e_{ijk} \quad (4.7)$$

where μ was the overall mean, α was the main effect of fraction, β was the main effect of grinder, γ_k was the main effect of severity of grinding, $(\alpha\beta)_{ij}$ was the interaction between the fraction and grinder, $(\gamma\beta)_{jk}$ was the interaction between severity of grinding and grinder, $(\alpha\gamma)_{ik}$ was the interaction between the fraction and severity of grinding, $(\alpha\beta)_{ij}$ was the interaction between the fraction and grinder, c_{ijk} was the plot error distribution and e_{ijk} was the subplot error distribution. Significance was defined as $P < 0.05$ and a trend was defined as $0.10 > P > 0.05$. In situations where there was an unequal number of samples between treatments, the largest standard error of the mean was used.

4.5 Results

Sorting wheat using the TriQ resulted in no difference in CP content among fractions ($P = 0.87$; Table 4.1) whereas for barley, a tendency was observed (Table 4.1; $P = 0.08$). Average CP content for the HCP barley fraction tended to be higher than the LCP fraction, with the unsorted fraction being intermediate. Regardless of grain, starch content was not different among fractions (Table 4.2; wheat, $P = 0.97$; barley, $P = 0.54$). No difference was observed in the PI between grinders for the unsorted wheat or barley (Table 4.3; $P < 0.98$ or $P < 0.89$, respectively). Grinding coarsely resulted in increased PI for both barley and wheat while grinding finely resulted in lower PI for both barley and wheat (Table 4.3; $P < 0.50$).

For barley, DGM and S_{gw} differed between grinders (Table 4.4; $P < 0.01$, $P < 0.01$ and $P = 0.03$, respectively). A significant interaction for severity of processing × grinder method was observed for barley DGM (Figure 4.1; $P < 0.01$) where roller mill processed barley had higher DGM than hammer mill processed barley. In addition, there was a significant interaction for severity of processing × grinder method for surface area, where roller mill processed barley surface area was less than hammer mill processed barley (Figure 4.2; $P < 0.01$).

Total gas production and DMD did not differ between fractions of barley but was affected by grinder and severity of processing (Table 4.5; $P < 0.01$). Total starch remaining after 12 h differed between the grinder types but was not affected by severity of processing (Table 4.5; $P < 0.01$ and $P = 0.37$, respectively). Starch digestibility after 12 h differed between grinder types but not with severity of processing (Table 4.5; $P < 0.01$ and $P = 0.39$, respectively). A

tendency was observed for TGP with the interaction of grinder type × severity of processing (Table 4.5; $P = 0.08$).

For wheat (Table 4.6), there was an interaction between type of grinder (roller mill vs hammer mill) and severity of grinding (coarse vs fine) for surface area ($P < 0.01$) and DGM ($P < 0.01$). Wheat DGM produced following roller milling was higher under coarse than fine grinding. In addition, hammer milling produced a higher surface area than roller milling for wheat (Figure 4.2). The surface area of hammer milled, finely ground barley differed between HCP and LCP, but neither differed from unsorted barley. In contrast, when a hammer mill was used, the DGM was higher with coarse grinding than with fine grinding (Figure 4.6). There was a three-way interaction for barley grain surface area between fraction, type of grinder and severity of grinding (coarse vs fine) (Figure 4.3; $P < 0.01$).

The number of particles produced for wheat under different methods of grinding differed between grinder type as well as with the severity of grinding, with roller milling producing a substantially lower number of particles for both fine and coarse grinding than hammer milling, and under hammer milling, a lower number of particles was produced by coarse grinding than by fine grinding ($P < 0.05$; Figure 4.5). Surface area for wheat grain (Figure 4.7) was similar under roller milling for the three fractions whereas, under hammer milling, HCP had the lowest surface area and the unsorted grain had the highest surface area.

Wheat TGP and DMD did not differ between fractions but did differ between grinder type and severity of processing (Table 4.7; $P < 0.01$). Total starch remaining after 12 h differed between types of grinder but not with severity of processing (Table 4.7; $P < 0.01$ and $P = 0.63$, respectively). Starch digestibility after 12 h differed between grinder type but not with severity of processing (Table 4.7; $P < 0.01$ and $P = 0.75$, respectively). A tendency was observed for DMD with grinder type × severity of processing × TriQ fraction (Table 4.7; $P = 0.09$).

4.6 Discussion

In commercial feed mills, grain is not presorted prior to grinding which is typically performed using either a hammer mill or a roller mill. Moreover, grinder settings are not adjusted

between batches of grain. Wheat or barley arriving at a mill can vary in individual kernel physical parameters as well as chemical composition. Adjustment of grinders is necessary because the variation in physical or chemical traits may result in over or under processing. Over processing of grain can result in digestive upsets or underutilization of nutrients (Dehghan-Banadaky et al. 2007). The NITS used in this project is designed as a tool for feed mills to presort grain into fractions based on predicted CP content. Differences in predicted CP content could result in differing nutritive profiles, physical characteristics and responses to grinding as well as digestibility. It was hypothesized that fractions produced by NITS would differ in digestibility and grinding characteristics (DGM and S_{gw}) and, thus, adjustment to grinding techniques could be used to optimize the digestibility of each fraction.

Significant differences in CP content exist between varieties for both wheat and barley (Alijošius et al. 2016; Guo et al. 2016; Rodehutscord et al. 2016). However, sorting wheat and barley based on predicted CP content in this trial with the TriQ resulted in fractions that were similar in CP content for wheat, and only slightly different for barley (Table 4.1). The TriQ was designed to sort grain into fractions that differ in CP content. Reasons for the failure to produce fractions that differed in CP content could be because the drum used in the TriQ did not permit a proper alignment of kernels to the NIT detector, or because the calibration used was not sufficiently accurate or precise to allow kernels having differences in CP content to enter their appropriate fraction.

According to the manufacturer of the drum, which rotates at 60 rpm, it has holes designed to allow individual kernels to settle where they will be detected by NITS 18 times for each kernel (Kautzman et al. 2015b; Kautzman et al. 2017). Although the number of kernels that were outliers is not determined by the instrument, the software indicated that this was a large portion. According to the manufacturer, outliers occur because the kernels did not orient correctly within the holes. Barley (Ahmad et al. 2010; Aghajani et al. 2011; Nair et al. 2011) and wheat (Nelson 2002; Al-Mahasneh and Rababah 2007) grains can vary considerably in kernel size. The variation in kernel size must be considered when choosing the drum. Prior to sorting with a TriQ, samples should be sorted using a mesh with screen size that will meet the requirements for the drum. This will permit a more homogenous kernel size entering the TriQ and thus reduce the

percent of outliers and increase the efficiency of sorting. The impact of sorting on actual CP in each fraction using such methodology has not been investigated and further work is needed.

The failure of the TriQ to separate individual fractions into different CP fractions also could be due to the calibration used. The calibrations are proprietary and thus could not be tested. Calibrations to predict CP content of wheat have previously been developed (Hansen et al. 2002; Pasikatan and Dowell 2004; Başlar and Ertugay 2011). These calibrations varied between samples and the NIR instrument used to obtain the spectrum. For barley, CP content at harvest was poorly predicted using NIR because of a weak correlation between the spectrum and CP content (Hansen et al. 2002). These findings were contrary to those of McCann et al. (2006) where predictions of *in vitro* ileal CP digestibility for barley using an NIRS had a coefficient of determination (r^2) of 0.97. These two studies were done using different methods of obtaining spectrum, as well as different sample sets. The difference in samples and method of analysis resulted in differences in calibrations and accuracy. Therefore, it is essential that the calibration be developed with samples that are similar to what is expected to be used within this calibration. Near infrared reflectance spectroscopy can be used to predict CP content but the calibration must be developed using samples that will represent the population that will be tested in the future. The failure of the TriQ to produce fractions that differed in CP content could be due to the samples used in developing the calibration not being based on the population that was assessed within this experiment.

Several reviews have examined the accuracy and precision of NIRS and potential issues (Chen and Sun 1991; Delwiche 1998; Givens and Deaville 1999; Workman Jr 1999; McClure 2003; Pasquini 2003). The TriQ uses a narrow spectrum. While this is not defined by the manufacturer, it will be between 750 nm to 2500 nm, as this is the range that NITS utilizes. Delwiche (1998) showed that using a wavelength between 1100 to 1400 nm produced a strong correlation between the spectrum and CP content for wheat.

Crude protein content and starch content in a grain are inversely related (Jenner et al. 1991) and there was no significant difference in starch content between the fractions for either wheat or barley. The content of starch and protein and how they interact will affect hardness and thus the particle size produced by grinding (Wu et al. 1990; Glenn et al. 1991). It is thus

expected that fractions similar in composition will have similar hardness and should produce a similar particle size after grinding. This can be verified in future studies by identifying the hardness of each fraction. Hardness of wheat is impacted by several factors, which include the adhesion of the protein matrix to the starch granules, protein matrix characteristics and CP content (Anjum and Walker 1991).

The interaction between grinder and severity of processing for particle size distribution (DGM, surface area and number of particles), indicated in Table 2.4 and Figure 2.1 for barley and Table 2.6 and Figure 2.6 for wheat, could be due to several factors. Regardless of processing severity, the hammer mill produced a greater proportion of fines than did the roller mill (Svihus et al. 2004). This also explains why fine or coarse grinding by hammer milling produced a lower DGM for barley and wheat than grinding by roller milling. The lack of difference between coarse and fine grinding by roller milling for barley could be due to the greater proportion of fiber in barley than in wheat (Svihus and Gullord 2002). A lower PI may be necessary for barley to observe a difference in particle size distribution for the type of roller mill used in this study. The three-way interaction observed in Figure 2.3 illustrates an interaction between fraction, method of grinding and severity of processing for surface area. Surface area produced by coarse grinding under roller milling was similar between all three fractions. This consistency could be explained by a higher proportion of fines across the fractions and, as described before, potentially the same hardness due to the same levels of starch and CP content between fractions. Overall, there were some differences in grinding properties between the fractions of barley; however, this is difficult to explain due to the absence of differences in protein and starch content.

Differences in physical and chemical traits could conceivably contribute to changes in nutrient digestibility. Nutrient digestibility can be estimated using several *in vitro* approaches. These approaches measure rate of nutrient digestibility as well as total digestibility. One of these approaches is to measure TGP using rumen fluid that provides the medium for microbial populations to act on the sample of interest, and thus allows the determination of total starch and total DM digestibility within a specified time (Krishnamoorthy et al. 2005). The TGP is measured for a specified period. It is a rapid approach allowing the assessment of many treatments (Mohammadian-Tabrizi et al. 2011). In addition, the TGP can be used to measure

starch digestibility by measuring the initial starch content versus the residue remaining. In out experiment, complete kinetics were not performed as the objective was to assess starch digestibility. More than 70% of the starch is digested within the rumen and thus prolonging digestibility to determine kinetics would not leave any starch to be measured (Hvelplund et al. 2009).

Digestibility's of starch and CP of the different fractions were not different, probably due to the lack of difference in starch and protein between the fractions, as well as the absence of differences in grinding characteristics. For both barley and wheat, TGP and DMD (Table 2.5 and Table 2.7) were affected by either the type of grinder or the severity of processing and to approximately the same degree for each. Total gas production of barley and wheat was higher when samples were ground using the hammer mill than when using the roller mill. This could be due to the presence of a larger proportion of fines obtained with hammer milling conditions than with roller milling; a higher S_{gw} was obtained for hammer milling than for roller milling (Table 4.4; 2.45 vs 2.35) indicating a greater distribution of particle size. Hammer milling produces more fines than roller milling (Thomas et al. 2018). Starch digestibility was affected by grinder type but not by the fraction produced by the TriQ. This can be explained by the lower DGM under either setting for the hammer mill when compared to the roller mill, and no difference between fractions. Increasing the severity of processing resulted in an increase in digestibility of starch, which is similar to the findings of Yang et al. (2001) where coarsely rolled (1.60 mm thick) barley had a lower digestibility than finely rolled barley (1.36 mm thick).

4.7 Conclusions

The use of a commercial NIT sorter designed to fractionate wheat or barley based on predicted CP content produced fractions that, when chemically tested, did not differ in CP or starch content, although barley fractions tended to be different in CP content. Grinding method and the degree of grinding changed DMD and TGP. The results indicated that the use of the NIT seed sorter would not provide an additional benefit to the feed industry. Lack of observed differences in chemical traits between fractions could explain the insignificant difference in digestibility. Future work will be needed to improve the accuracy of CP content prediction under commercial settings.

4.8 Next stage

The use of NITS did not provide a difference in digestibility for ruminants. Such a tool could nevertheless produce fractions with different amino acid profiles. This requires further investigation in monogastrics. The objective of the next study was to investigate whether fractions produced using NITS that predicts CP content will have different energy and protein digestibility using swine as the monogastric model. Additionally, it is necessary to identify if amino acid digestibility will be affected by the fraction being digested and if this will interact with the temperature used within the conditioner prior to pelleting the feed.

4.9 Acknowledgement

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4.10 Conflict of interest

None

Table 4.1. Average crude protein (CP) percentage of wheat[z] and barley[z] grain fractionated by TriQ into two fractions based on predicted CP, (high CP (HCP), low CP (LCP) and the initial unsorted grain (UNS).

	Fraction (%DM)				
	HCP	LCP	UNS	SEM[y] (%)	*P value*
Wheat	14.22	13.46	13.78	1.016	*0.87*
Barley	11.37	10.41	10.91	0.266	*0.08*

[z]Crude protein determined by using the combustion approach (AOAC 2005) to determine percent nitrogen multiplied by a factor of 5.83.
[y]Standard error of the mean.

Table 4.2. Average starch content (%) of wheat and barley grain fractionated by TriQ into two fractions based on predicted crude protein (CP), (high CP (HCP), low CP (LCP) and the initial unsorted (UNS) grain).

	Fraction (%)				
	HCP	LCP	UNS	SEM[z]	*P value*
Wheat	56.33	56.79	56.56	1.256	*0.97*
Barley	53.31	53.19	54.16	0.667	*0.54*

[z]Standard error of the mean.

Table 4.3. Processing index (PI; %) of unsorted fractions of five sources of wheat and barley grain obtained with different grinders and grinding settings.

	[z]Source (%)					Grinder (%)				Severity of processing (%)				P-value
	1	2	3	4	5	Hammer	Roller	SEM	P-value	Fine[x]	Coarse[w]	SEM	*P-value*	*Grinder *size*
Wheat	79.89	82.66	79.73	77.36	85.78	81.11	81.06	1.068	0.98	79.37	83.66	1.068	*0.01*	*0.76*
Barley	79.40	79.61	69.51	82.24	80.03	77.95	78.36	1.987	0.89	72.30	84.02	2.807	*0.01*	*0.87*

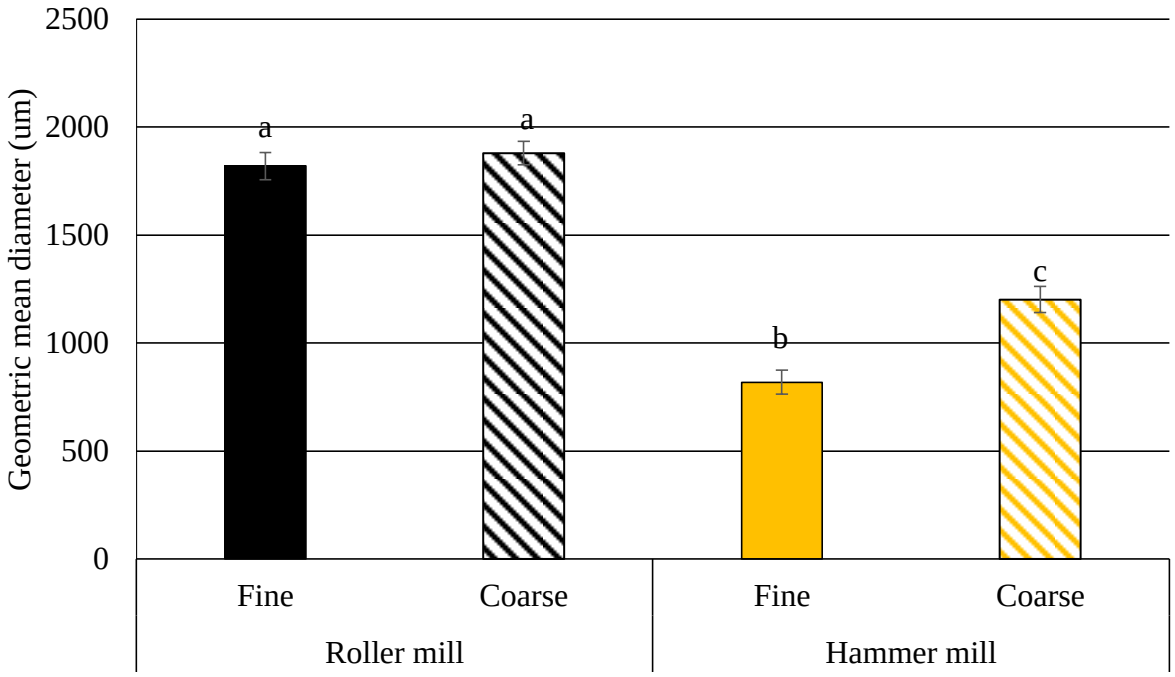
[z]Source represents the different batches purchased within western Canada.
[y]Standard error of the mean.
[x]Grinding performed with a hammer mill using a screen of 0.188mm.
[w]Grinding performed with a hammer mill using a screen of 0.375mm.

Table 4.4. Average geometric mean diameter (DGM), standard deviation of DGM (S_{gw}), surface area (Area) and number of articles per gram (*N*) of barley grain fractionated by TriQ into two fractions based on predicted CP, (high crude protein (HCP), low crude protein (LCP) and the initial unsorted (UNS) grain) using different grinders and severity of grinding.

	Fraction[z]				Grinder			Severity of grinding			Fraction	Grinder	S[y]
	LCP[u]	HCP[t]	UNS[s]	SEM[r]	Hammer	Roller	SEM	Coarse	Fine	SEM		*P value*	
N	16710	340894	29995	125987	250597	7802	97789	17778	240622	97925	*0.11*	*0.08*	*0.11*
Area (mm^2)	50.43	56.52	57.04	2.380	71.37	37.96	1.829	47.71	61.62	1.830	*0.10*	*0.01*	*0.01*
DGM (mm)	1508.64	1426.90	1352.18	53.478	1009.61	1848.86	41.509	1539.64	1318.83	41.567	*0.08*	*0.01*	*0.01*
S_{gw} (mm)	2.29	2.43	2.42	0.065	2.45	2.31	0.047	2.42	2.34	0.046	*0.23*	*0.03*	*0.18*

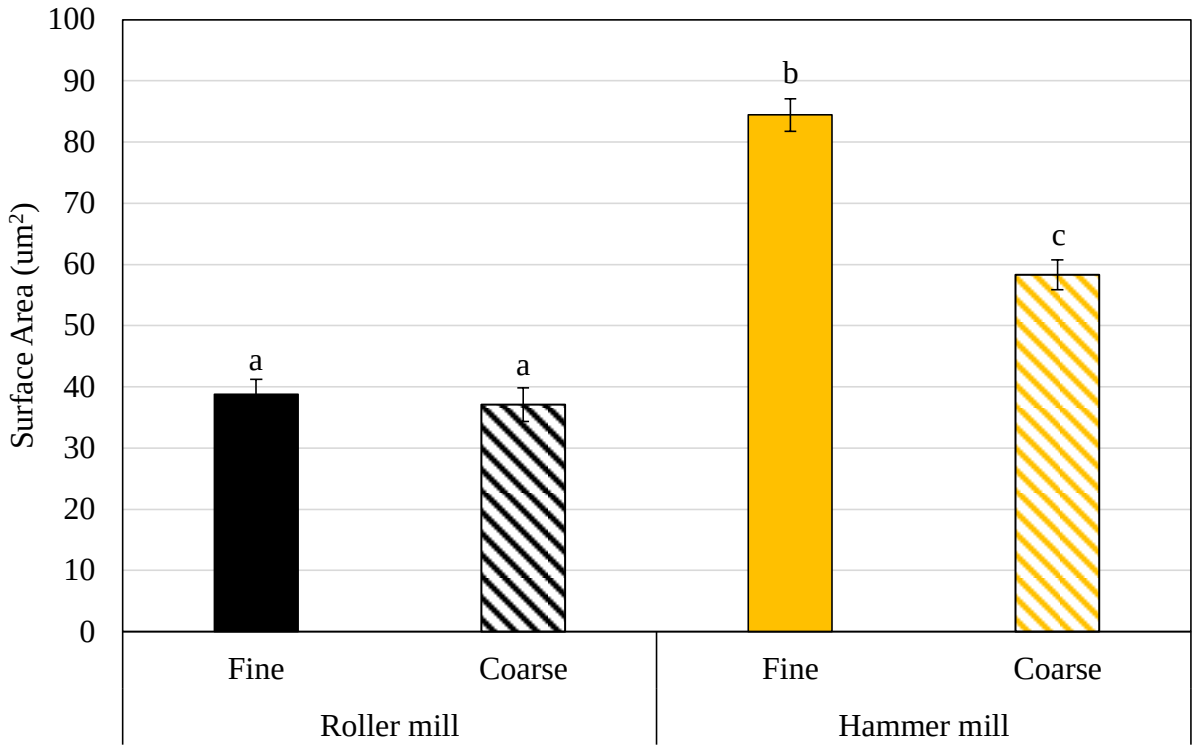
Note: Three-way interaction had a P > 0.10 for all variables assessed except for surface area which had a P < 0.048 and Grinder × Severity of grinding had a P > 0.10 except for Area and DGM where P < 0.01.

[z]Fraction represents the different fractions produced from each source of grain using the single kernel near infrared transmittance spectroscopy BoMill TriQ.
[y]Severity of grinding (Coarse versus fine).
[x]Fraction × Severity of grinding.
[w]Grinder × Severity of grinding.
[v]Fraction × Severity of grinding × grinder.
[u]Low crude protein fraction produced by the BoMill TriQ representing 30% of the lowest protein.
[t]High crude protein fraction produced by the BoMill TriQ representing 30% of the lowest protein.
[s]Unsorted fraction representing the initial grain prior to it being sorted by the BoMill TriQ.
[r]Standard error of the mean.



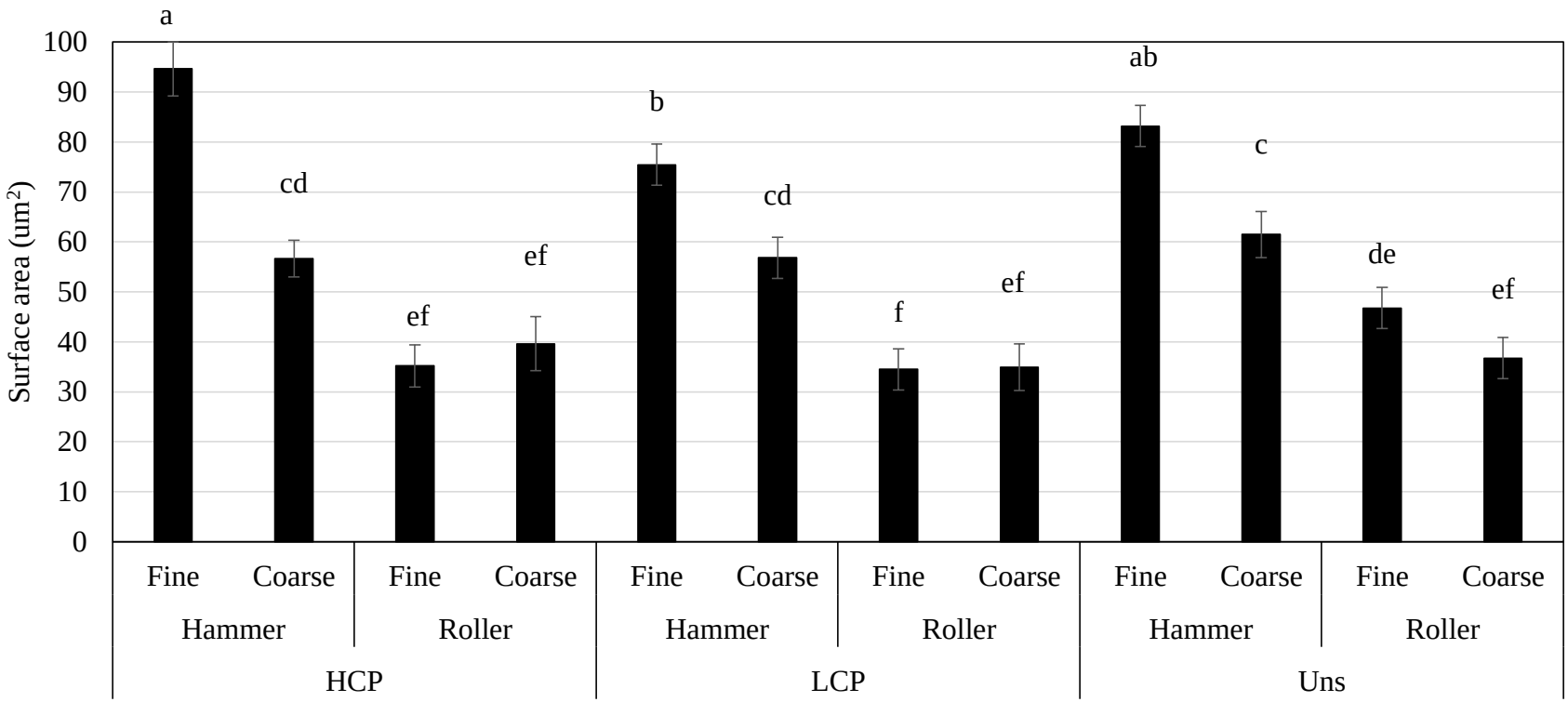
Different lowercase letters indicate a significant difference (P < 0.05).

Figure 4.1. Interaction between method of grinding and severity of grinding (coarse or fine) on DGM of barley. Grinding was performed using either a hammer mill with a screen size of 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.



Different lowercase letters indicate a significant difference (P < 0.05)

Figure 4.2. Interaction between method and severity of grinding on average surface area (um^2) of barley. Grinding was performed using either a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.



Different lowercase letters indicate a significant difference (P < 0.05).

Figure 4.3. Three-way interaction between method of grinding, severity of grinding and predicted CP fraction (sorted by single kernel near infrared transmittance spectroscopy) for surface area of the final ground particles for barley grain. Grinding was performed using either a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.

Table 4.5. *In vitro* gas production from barley sorted by single kernel near infrared transmittance spectroscopy sorter into three fractions based on predicted crude protein (CP) (low CP (LCP), high CP (HCP), initial grain prior to grinding (UNS)) ground either using a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the unsorted fraction obtained with the hammer mill.

	Fraction[z]				Grinder[y]			Severity of grinding			*P-value*	
	LCP[t]	HCP[s]	UNS[r]	SEM[q]	Hammer	Roller	SEM	Coarse	Fine	SEM	Grinder	Severity of grinding
TGP[x] (mL)	43.94	42.35	38.71	3.004	59.36	23.97	1.987	35.91	47.42	1.982	*0.01*	*0.01*
DMD[w] (%)	34.29	31.67	32.78	1.616	41.84	23.98	1.042	29.64	36.18	1.039	*0.01*	*0.01*
STARDIG[u] (%)	35.37	35.10	36.95	2.581	25.85	45.76	1.927	36.98	34.63	1.950	*0.01*	*0.39*

Note: Two-way interaction for grinder × severity was greater than P > 0.10 except for TGP which has a P = 0.08.
Three-way interaction had a P > 0.10 for all variables assessed. Fraction had a P>0.10
[z]Fraction represents the different fractions produced from each source of grain using a BoMill TriQ.
[y]Grinder.
[x]Total gas production (mL).
[w]Dry matter digestibility (%).
[u]Starch digested in 12 hours (%).
[t]Low crude protein fraction produced by the BoMill TriQ from each fraction.
[s]High crude protein fraction produced by the BoMill TriQ from each fraction.
[r]Unsorted represent the initial source of grain prior to sorting using the BoMill TriQ.
[q]Largest standard error of the mean.

Table 4.6 Average DGM, standard deviation of DGM (S_{gw}), surface area (Area) and number of particles per gram (N) of wheat grain fractionated by the BoMill TriQ into two fractions based on predicted CP, (high crude protein (HCP), low crude protein (LCP) and the initial unsorted (UNS) grain) using different grinders and severity of grinding.

	Fraction[z]				Grinder[y]			Severity of grinding			*P-value*			
	LCP	HCP	UNS[x]	SEM[w]	Hammer[u]	Roller[t]	SEM	Coarse[s]	Fine[r]	SEM	Grinder	S	G×S[q]	F×G[p]
N	25652	18875	49286	12003	61385	1157	8592.2	16284	46257	8513.3	*0.01*	*0.01*	*0.01*	*0.06*
Area (mm^2)	44	43	53	3.71	71	23	2.4226	38.83	54.98	2.41	*0.01*	*0.01*	*0.01*	*0.01*
DGM (mm)	1704	1690	1585	49.85	2287	1033	34.706	1825.6	1493.82	34.44	*0.01*	*0.01*	*0.05*	*0.21*
S_{gw} (mm)	2	2	2	0.06	2	2	0.043	1.92	2.02	0.04	*0.01*	*0.04*	*0.96*	*0.29*

Note: Three-way interaction had a P > 0.10 for all variables assessed except for standard deviation of DGM

[z]Fraction represents the different fractions produced from each source of grain using the single kernel near infrared transmittance spectroscopy BoMill TriQ.

[y]Grinder.

[x]Unsorted represent the initial source of grain prior to sorting using the BoMill TriQ.

[w]Largest standard error of the mean.

[u]Hammer mill grinder.

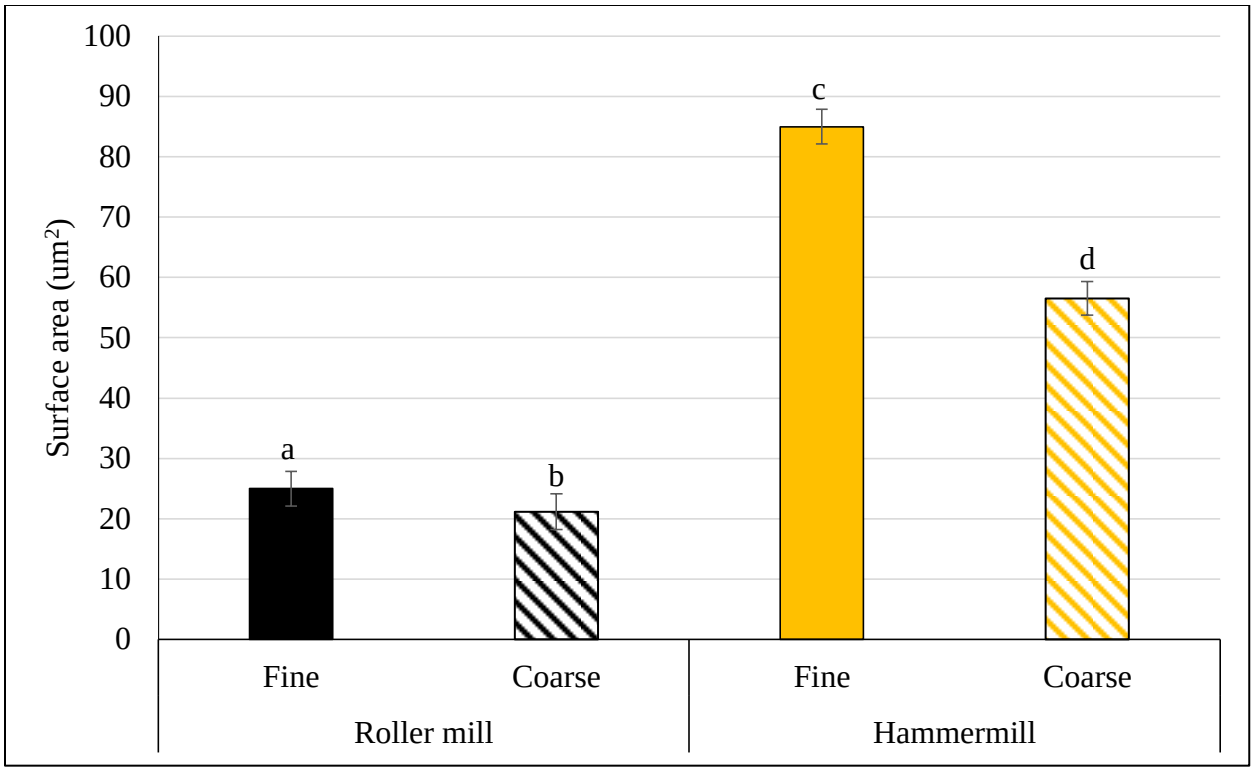
[t]Roller mill grinder.

[s]Coarse grinding.

[r]Fine grinding.

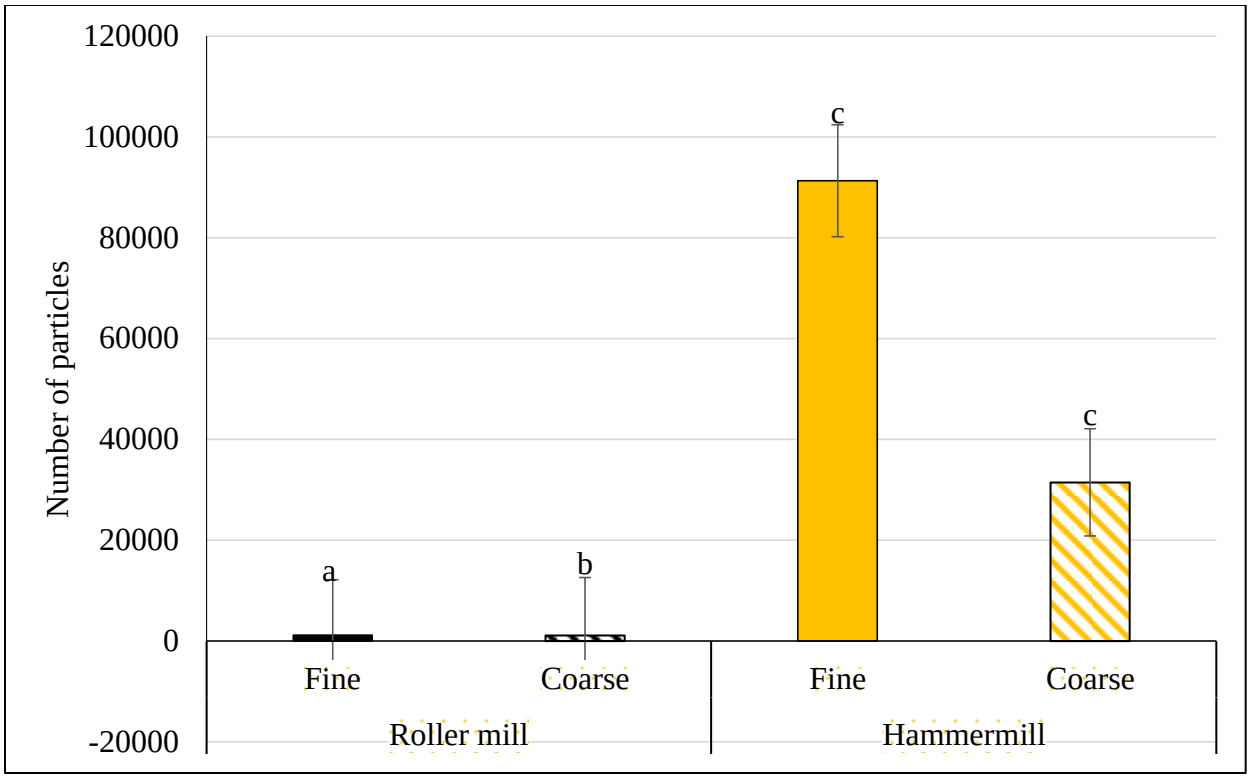
[p]Grinder × severity of grinding.

·[q]Fraction × grinder



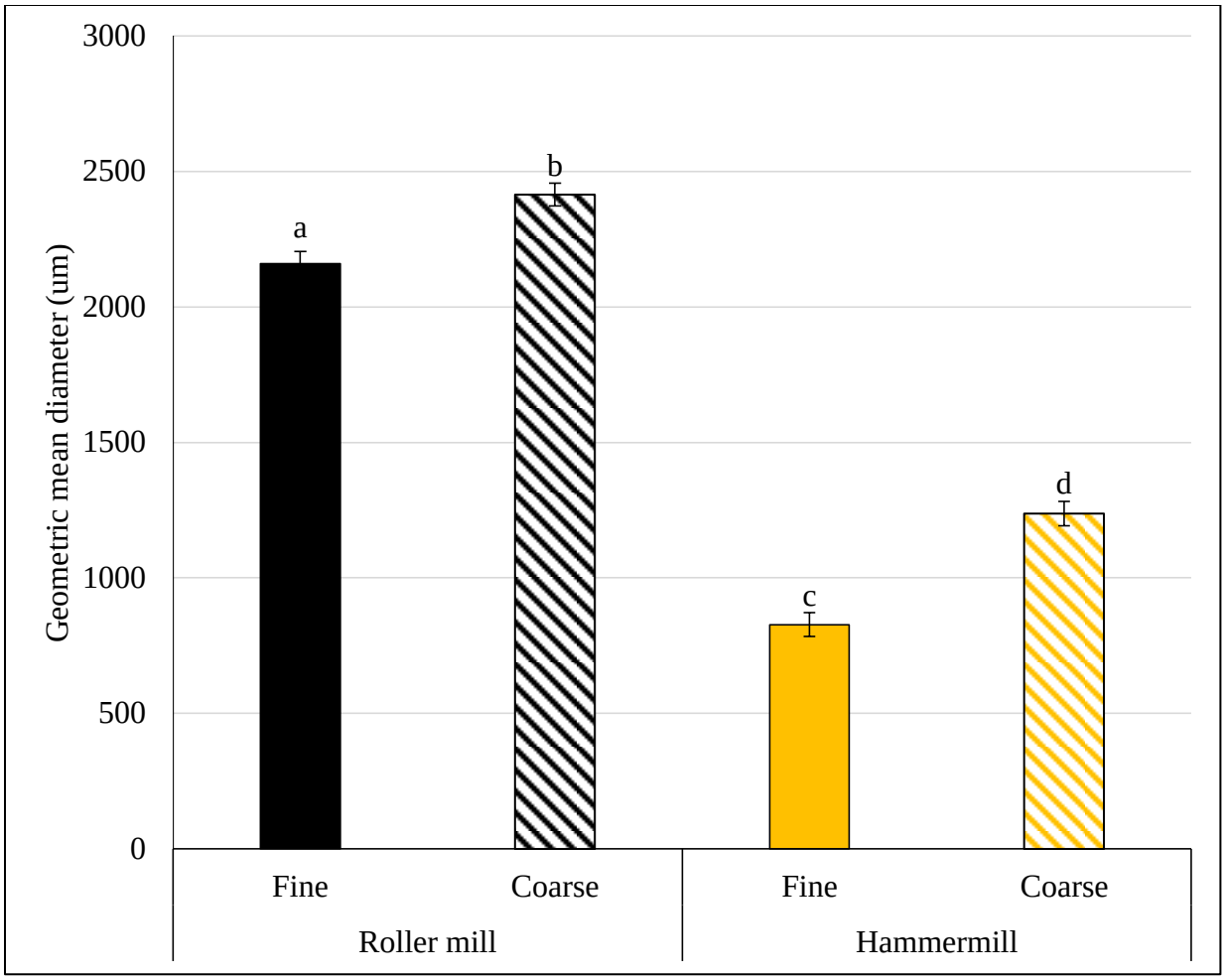
Different lowercase letters indicate a significant difference (P < 0.05)

Figure 4.4. Interaction between method and severity of grinding on wheat surface area (mm^2). Grinding was performed using a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.



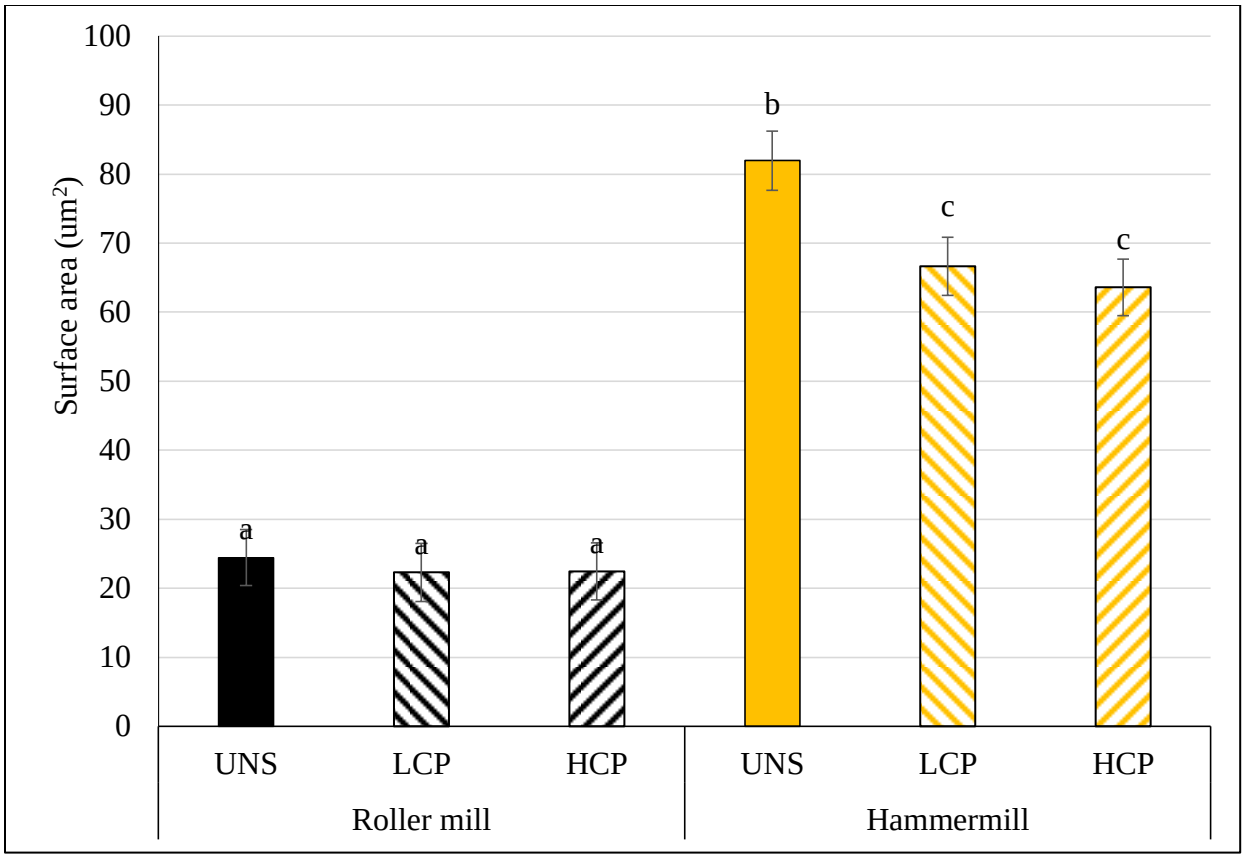
Different lowercase letters indicate a significant difference ($P < 0.05$).

Figure 4.5. Interaction between method and severity of grinding (coarse versus fine) on the number of particles for wheat. Grinding was performed using a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.



Different lowercase letters indicate a significant difference (P < 0.05).

Figure 4.6. Interaction between method of grinding and severity of grinding on DGM of the final ground particles for number of particles per gram of wheat grain. Grinding was performed using a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.



Different lowercase letters indicate a significant difference (P < 0.05).

Figure 4.7. Interaction between grinding and predicted CP fraction (sorted by single kernel near infrared transmittance spectroscopy into three fractions based on predicted CP) on surface area of the final ground particles of wheat grain. Grinding was performed using either a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the initial bulk sample.

Table 4.7. *In vitro* gas production of wheat sorted into three fractions based on predicted crude protein (CP) (low CP (LCP), high CP (HCP), initial grain prior to grinding (UNS)) ground using a hammer mill with the diameter of the round holes in the screen being 0.375 mm (coarse) or 0.188 mm (fine) or a roller mill to produce the same processing index as the unsorted fraction obtained with the hammer mill

	Fraction[z]				Grinder[y]			Severity of grinding			*P-value*	
	LCP [t]	HCP[s]	UNS[r]	SEM[q]	Hammer	Roller	SEM	Coarse	Fine	SEM	Grinder	Severity of grinding
TGP[x] (mL)	47.33	46.21	43.86	1.861	63.78	27.82	1.483	42.72	48.88	1.475	*0.01*	*0.01*
DMD[w] (%)	35.55	33.35	33.93	1.044	42.31	26.25	0.818	32.21	32.21	0.822	*0.01*	*0.01*
STARDIG[u] (%)	44.91	44.40	42.80	0.016	38.00	50.00	0.013	44.33	43.74	0.013	*0.01*	*0.75*

Note: Three-way interaction had a P > 0.10 for all variables assessed except DMD where P = 0.09.

[z]Fraction represents the different fractions produced from each source of grain using a BoMill TriQ.
[y]Grinder.
[x]Total gas production (mL).
[w]Dry matter digestibility (%).
[v]Total starch remaining (%) at 12 hours.
[u]Starch digested in 12 hours (% based on DM).
[t]Low crude protein fraction produced by the BoMill TriQ from each fraction.
[s]High crude protein fraction produced by the BoMill TriQ from each fraction.
[r]Unsorted represent the initial source of grain prior to sorting using the BoMill TriQ.
[q]Largest standard error of the mean.

5 THE EFFECT OF SORTING WHEAT OR BARLEY, BASED ON THE PREDICTED CRUDE PROTEIN OF INDIVIDUAL SEEDS, ON TOTAL TRACT DRY MATTER AND ILEAL AMINO ACID DIGESTIBILITY IN PIGS

5.1 Abstract

Variability in the physiochemical profile of cereal grains represents a challenge for the livestock industry. Current nutrient values are based on sample averages, ignoring the inherent variation in a sample. However, it is not known if sorting grain based on specific, defined criteria would result in fractions which differ in feed quality. This experiment was designed to determine if fractions obtained by an instrument calibrated to separate kernels based on predicted crude protein (CP) content, differ in their dry matter (DM) and amino acid digestibility (AAD) and response to hydrothermal processing. The BoMill TriQ (TriQ), which uses near infrared transmittance spectroscopy (NIT) to estimate the CP content of individual seeds, was used to separate individual kernels of wheat or barley into low and high CP fractions (LCP and HCP). Treatments, arranged as a 2 x 2 x 2 factorial included two grains (wheat or barley), two fractions (LCP vs HCP) and two processing temperatures (low versus high temperature pelleting) plus a nitrogen free diet to estimate endogenous losses. Treatment diets were fed to 16 ileal-cannulated pigs in three blocks providing an *n* of 6 per treatment. Data were analyzed independently by grain type including the effects of fraction, hydrothermal treatment and their interactions. Most amino acids for either wheat or barley did not differ between fractions in digestibility. The lack of difference for most of the parameters assessed could be attributed to similarity in chemical composition between the fractions. The similarity between the fractions was due to the failure of the TriQ to separate the sources used in this study into fractions that differed in CP content.

Keywords: Near infrared transmittance spectroscopy, grain variability, wheat, barley, swine, hydrothermal, amino acid digestibility.

5.2 Introduction

Feed cost in the pork industry often exceeds 50% of the total production cost (Coulibaly 2009). This cost can be reduced by ensuring swine are fed diets which closely match their nutrient requirements. Tailoring the diet of an animal to meet its requirements improves efficiency and reduces feed wastage (Pomar et al. 2009). Minimizing nutrient variability of ingredients is required to feed diets to pigs which closely match nutrient requirements. This can be accomplished by minimizing variation within ingredients. Different varieties and management practices result in chemical and physical variation of grains (Ball et al. 2013). For example, the incorporation of different levels of nitrogen fertilizer on different wheat varieties resulted in test weights that ranged from 59 kg hl^{-1} to 78 kg hl^{-1}, thousand grain weight from 21.7 g to 60.8 g, and *in vitro* viscosity ranging from 4.3 mPa to 44.0 mPa (Ball et al. 2013).

McCracken et al. (2002) analyzed 24 varieties of wheat grown in three locations in one year and found test weight ranged from 63.2 to 77.1 kg hl^{-1}, while CP content ranged from 115 to 147 g kg^{-1} DM. Barley CP content can vary from 8 to 15% (Kirkman et al. 1982). The starch content of barley can vary from 51.3 to 67% (Per et al. 1985; Holtekjolen et al. 2006).

To determine the nutrient content within a grain load, multiple samples can be taken and analyzed. This is typically done by wet chemistry or near infrared reflectance spectroscopy (NIRS) which has been calibrated using wet chemistry. Wet chemistry is destructive to the sample, has a turnaround time that can be from a few hours to a few days, and is costly, whereas NIRS can provide an instant measurement and the sample is not destroyed, allowing it to be incorporated into an in-line system (Gradenecker 2003). Currently, the majority of NIRS or near infrared transmittance spectroscopy (NITS) technology is based on analyzing a sample and using the spectrum of that sample to determine the chemical composition based on previously established calibrations.

The BoMill TriQ (TriQ) is a single kernel NITS seed sorter unit with a built-in calibration to predict CP content. The TriQ can sort grain into fractions differing in CP content. It was hypothesized that these fractions would differ chemically and that the chemical

differences would affect digestibility. It was also hypothesized that digestibility would be affected by the interaction between chemical variation and hydrothermal processing temperature. The objective of this investigation was to determine if fractions obtained by an instrument calibrated to separate kernels based on predicted CP content would differ in DM and AAD and respond differently to hydrothermal processing.

5.3 Materials and Methods

The experimental protocol was approved by the University of Saskatchewan committee on animal care and supply for compliance with the principles of the Canadian Council on Animal Care (2009). All animal trials were conducted at the Prairie Swine Centre, Saskatoon, SK and all grain sorting and feed production utilized the Canadian Feed Research Centre (North Battleford, SK).

5.3.1 Grain sourcing and fractionation

Two sources of independent feed grade barley and wheat, from separate locations in western Canada, were purchased. Grains were sampled upon arrival. Grains were analyzed for mycotoxins (Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, SK). Mycotoxin tested include *ergocomine*, e*rgocristine*, e*rgocryptine*, e*rgosine*, *3-acetyl-deoxynivalenol*, *15-acetyl-deoxynivalenol*, *α-zearalenol*, d*eoxynivalenol*, *diacetoxyscirpenol*, *HT-2 toxin, nivalenol*, *ochratoxin A*, *T-2 toxin*, *β-zearalenol*, *zearalenone* and *aflatoxin B1*. The wheat and barley were then cleaned using a seed cleaner AS4 model 6048 Air and Seed Cleaner (Flaman, Saskatoon Canada) which removes unwanted material using a set of vibrating sieves. The grain was then sorted by the BoMill TriQ (TriQ; BoMill AB, Vintrie, Sweden).

The TriQ contains a drum with 256 rows with 88 laser etched pockets within each row. These pockets allow individual kernels of wheat or barley to be positioned for reading by 18 NIT detectors. The built-in calibration to predict CP content was based on a limited spectrum (proprietary). The initial process of sorting the grain required the production of 10 fractions as described by Kautzman et al. (2015b). These 10 fractions contained an equal number of kernels and represented a normal distribution curve. Fractions 1 and 10 contain kernels identified by the system as outliers. These are defined by the system as kernels that are too large to fit into a

pocket or kernels that were too small, or two or more kernels trapped within one pocket. An outlier may also be a kernel that does not enter the pocket in the correct orientation or the predicted CP content is outside the range of the calibration. The system does not indicate to the user the reasons for the outliers.

Sorting was performed following the calibration. The TriQ was set to produce three fractions. The fractions were LCP (lowest 30% of the predicted CP), medium CP plus outliers and a third HCP fraction (highest 30% of the predicted CP). For each of the fractions and the initial unsorted grain, samples were collected and stored at -20°C until further analysis. Production rate of the TriQ was determined by sorting for 3 min and then weighing the fractions.

5.3.2 Diets and Hydrothermal Processing

Fractions produced by the TriQ were ground using a hammer mill operating at 3600 rotations per minute (RPM) through a 0.318 cm screen (G.J. Vis Model: VISHM2014, Ag Growth International Inc, Winnipeg, Manitoba, Canada). The diets were formulated to maximize grain inclusion to meet requirements for 40 kg pigs. Each of these fractions were pelleted under either high or low temperature. In addition, a nitrogen free (N-free) diet was used in the seventh period to determine basal endogenous nitrogen losses. The N-free diet was not pelleted.

Diets were mixed with a twin shaft paddle mixer (UAS-Muyang-Model: SLHSJ1, Shenzhen, Guangdong, China) for 90 seconds and then discharged to the pelleter. Pelleting was performed using a triple barrel conditioner (Buhler Conditioning system, Buhler, Uzwil, Switzerland) allowing a maximum retention for 120 s prior to entering the pelleter (UAS-Muyang model: MUZL35011, Shenzhen, Guangdong, China). Conditioning was performed at a high (85°C) or a low temperature (70°C). Temperature was maintained within these specifications by adjusting the steam entering the conditioner. Pelleting was performed using a 4.0 mm die with a 35% perforated die with two rolls each having a diameter of 150 mm. The pelleter die had a pore width of 3.97 mm and a thickness of 540 mm. Pellets exiting the pelleter were screened using a rotary-shaker screen (UAS-Muyang Model: SFJH80X3C, Shenzhen, Guangdong, China) to remove fines. Pellets were sampled and analyzed for pellet durability index (PDI). Pellet durability index was tested using a Homan Pellet Tester (HPT; Holman

Chemical Ltd, Norfolk, United Kingdom). The protocol was adapted from Wood (1987) where 100 g of pellets were placed into the HPT. The HPT pushes air through the 2-mm screen where the pellets resides causing the fines to separate from the intact pellets. Pellets remaining were weighed. Durability was determined by the average of three repeats. The PDI had to be within 90% ± 5% for the pellets were to be accepted.

5.3.3 Animals and housing

All pigs were PIC genetics (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada). Eighteen barrows weighing 25 kg ± 2 kg were transferred to individual 1.5 x 1.5 m metabolism crates. The flooring of the crates was composed of plastic-coated, expanded metal and the walls were PVC with a small window where pigs in adjacent pens had visual, but not physical contact. To ease acclimation, pigs were provided a variety of enrichments each day. Room temperature was maintained at 25°C with an automated ventilation system.

Cannulas were surgically inserted into the terminal end of the ileum, 5 cm from the ileo-cecal sphincter (Li et al. 1993). Eighteen pigs were cannulated over five days, two extra pigs were cannulated in case of infection, feed refusal, or any other problem requiring an animal to be removed from the experiment. Following surgery, pigs were given analgesics and antibiotics as per the veterinarian's instructions and crates were equipped with heat lamps for one week.

Pigs were fed a standard commercial diet prior to surgery. The initial allotment post-surgery of 100 g was increased each day for 10 d until they were allocated to treatment diets. Treatment diets were provided at 3 × maintenance (110 kcal DE kg^{-1} body $weight^{0.75}$; NRC 2012). The daily feed allotment was divided into two meals at 0900 and 1600 h. Remaining feed was removed after two hours and, if this exceeded 10%, the allotment was reduced by 10%. The mash N-free diet was fed in a 1 to 1 ratio with water. Water was freely available in all pens from a nipple drinker.

The first six periods were 8 d; a 4-d diet acclimation followed by a 4-d ileal collection period. The seventh experimental period was 10 d; a 6-d acclimation to the N free diet, followed

by four days for ileal collection. Ileal collection started in the morning of d 5 by securing a plastic bag containing 15 mL of 5% formic acid around the open cannula barrel of each pig using a rubber band. The last bag was removed at 1800 h on day 8. Bags were changed throughout the day when deemed necessary. Blood samples were obtained 30 min prior to and 45 minutes after the morning feeding on d 4 via cranial vena cava venipuncture using an 8-mL vacuum tube. The tubes were centrifuged for 15 min at 830 *g* and the serum was collected and stored at -20°C.

5.3.4 Analysis

5.3.4.1 Water hydration capacity

Water hydration capacity was determined using a protocol adapted from Draganovic et al. (2011). Each of the treatments was ground using a hammer mill with either a 1/4 inch or a 1/8-inch screen. Five grams of the ground treatment were dispersed in 25 mL of distilled water in a 50 mL centrifuge bottle. Bottles were then agitated for 10 min in a water bath set to either 25°C, 65°C or 75°C and then centrifuged at 4000 *g* for 30 min. The supernatant was decanted, each bottle was weighed and the WHC was calculated based on Equation 5.1.

$$\text{WHC}\ (\%) = \frac{W_0 - \Delta W}{W_0} \times 100 \qquad (5.1)$$

where W_0 was the final tube weight with 5 grams of materials and ΔW was the change in weight after centrifugation.

5.3.4.2 Viscosity

The determination of wheat and barley viscosity (performed in triplicate unless the CV was greater than 5% for which a fourth replica was than performed) was based on protocols adapted from Campell et al. (1989) and Scoles et al. (1993), respectively. Samples were ground through a 1 mm screen and approximately 200 mg barley or 500 mg wheat, was placed into 12 mm × 75 mm glass tubes. One mL of 0.1 M KCl buffer was added to barley and 1 mL of 0.1 N Na acetate was added to wheat. Both were then incubated in a shaking water bath set at 40^{o}C for 30 min.

The contents were poured into 1.8 mL micro centrifuge tubes kept on ice, until they were

centrifuged for 5 minutes at 18,900 *g* at room temperature. The tubes were then returned to ice and viscosity was determined using a Brookfield cone-plate viscometer (Model LVDV-111, Brookfield Engineering Laboratories Inc, Middleboro, Massachusetts, USA). The Brookfield cone-plate viscometer temperature was maintained at 40^{o}C with the following setting: 12 rpm; shear rate 90 s^{-1} and at 7 rpm; shear rate 52.55 s^{-1}. For each treatment, three replicates were performed, unless CV was higher than 5% when a fourth replica was performed.

5.3.4.3 Porcine C-peptide

The Porcine C-peptide concentration of the pig serum was determined at Prairie Diagnostic Services (University of Saskatchewan, Saskatoon Saskatchewan, Canada) using a commercial ELISA kit (Mercodia Porcine C-peptide, catalog number 10-1256-01; Mercodia AB, Uppsala, Sweden). The standards used include Porcine C-peptide hormone, 0.05 to 3.13 mg/mL). The mouse monoclonal antiporcine antibody was provided by the ELISA kit. Unbound antibodies were removed, and bound ones reacted with 3,3-5-5 tetramethylbenzidine which would result in a change in colour followed by a colorimetric reading at 450 nm. The complete protocol as indicated by the manufacturer was followed.

5.3.4.4 Chemical analysis and digestibility of protein and energy.

The DM content of the ingredients and diets were determined using the oven method (AOAC 930.15). The ileal digesta were freeze dried. Samples were then ground through a 1 mm screen in a Restch Mill (ZM1, Newton, PA, USA). The following analyses were conducted at the University of Saskatchewan, College of Agriculture and Bioresources, Department of Animal and Poultry Science. Percent nitrogen (N) was determined by the combustion method (AOAC 968.06) using a LECO FP528 (St Joseph MI, USA). Crude protein content was calculated as N × 6.25 for the diet and ileal samples. When determining wheat or barley CP content, it was calculated as N × 5.83. Gross energy of each sample was determined by combustion in a bomb calorimeter (6400 Automatic Isoperibol Calorimeter, Parr Instrument Company, Moline, Illinois, USA) by measuring the heat of combustion.

Titanium oxide in the diet and digesta was determined using modifications of Jagger et al. (1992), Short et al. (1996), Titgemeyer et al. (2001) and Myers et al. (2004). One CT-37 Kjeldahl

tablet (Fisher Tab K301-1000) was placed into a Kjeldahl tube with 0.5 g of the sample. Each tube was digested with concentrated sulfuric acid (H_2SO_4, d = 1.84 g cm^{-3}) at 420°C for 2 h. The tubes were then filtered into a 100-mL tube and distilled water was added to wash any remaining material. This was then transferred into a beaker. Five mL of each sample obtained from the beaker, and 0.2 mL hydrogen peroxide was added to the 16 mm × 100 mm size test tubes. The standards, 0, 2, 4, 6, 8 and 10 mg TiO_2 were added to separate 16 × 100 mm test tubes with 0.2 mL hydrogen peroxide. Absorbance was measured at 408 nm. The standard, 0 mg of TiO_2 was used to zero the instrument. A linear regression was developed between absorbance and mg of TiO_2.

Starch content of the initial fractions was determined using the Megazyme kit (Megazyme International Ireland, Wicklow, Ireland). This protocol is based on enzyme hydrolysis of α-linked glucose (Equations 5.2 and 5.3). The protocol required the gelatinization of all starch within the sample, followed by enzymatically degrading it into glucose. A quinoneimine dye was added and glucose was determined calorimetrically at 510 nm.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2 \quad (5.2)$$

$$H_2O_2 + \text{Phenol} + 4 - \text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H2O \quad (5.3)$$

The coloured complex with quinoneimine was directly proportional to the concentration of glucose. The absorbance at 510 nm was used to calculate percent starch according to the formulae indicated in Equations 5.4, 5.5 and 5.6.

$$\text{Starch, \%} = \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \quad (5.4)$$

$$\text{Starch \%} = \Delta A \times \frac{F}{W} \times FV \times 0.9 \quad (5.5)$$

$$F = \frac{100\ (\text{ug of D}-\text{glucose})}{\text{absorbance for 100 ug of glucose}} \quad (5.6)$$

Where ΔA is the absorbance read against the blank reagent, F is conversion of absorbance to

glucose as defined in Equation 5.4, FV is final volume, W is the weight in mg of the ground material, $\frac{100}{W}$ is the factor to express starch as a percent of the weight of the material, and $\frac{162}{180}$ is used to adjust from the free D-glucose to anhydro D-glucose which occurs in starch.

The Ca, P, Na, ADF and NDF content were analyzed by a commercial laboratory (Central Testing Laboratory Ltd Winnipeg, Manitoba, Canada). Amino acids were determined at the University of Manitoba Faculty of Agricultural and Food Science, Department of Animal Science using AOAC method 994.12, based on hydrolysis of protein into amino acids followed by detection with HPLC. Tryptophan was determined independently by the University of Manitoba, Faculty of Agricultural and Food Science Department of Animal Science using AOAC method 994.12 (AOAC 1997).

5.3.4.5 Digestibility

Standardized ileal digestibility was calculated by Equations 5.7 to 5.9.

$$\text{Apparent Digestibility (\%)} = 100 - \left[100 \times \left(\frac{\%TI}{\%Td}\right) \times \left(\frac{\%NT}{\%Nd}\right)\right] \quad (5.7)$$

$$\text{Total IAA}_{\text{end}} = \text{AA}_{\text{digesta}} \times \frac{\text{M}_{\text{diet}}}{\text{M}_{\text{digesta}}} \quad (5.8)$$

where total IAA_{end} was the basal endogenous loss of an amino acid in g kg^{-1} of DM, AA digesta was the concentration of that amino acid in the ileal digesta (g kg^{-1} of DM), M_{diet} was the marker concentration in the diet (g kg^{-1} of DM), and $M_{digesta}$ was the marker concentration in digesta (g kg^{-1} of DM).

$$\text{Standardized ileal Digestibility (\%)} = \text{Apparent digestibility} + \left[\left(\frac{\text{total IAA}_{\text{end}}}{\text{AA}_{\text{diet}}}\right) \times 100\right] \quad (5.9)$$

where %Ti was % TiO_2 in the ileal digesta, %Td was % TiO_2 in the diets, %NI was % Nutrient in the ileal digesta, and %Nd was % Nutrient in the diet.

5.3.5 Statistics

Each experiment (wheat and barley) was analyzed using a randomized block design with

2 sources × 2 fractions × 2 temperatures of pelleting in a factorial arrangement. Treatment diets were fed to two groups of eight pigs in six blocks, for an $n = 6$.

A CRD design, as indicated below in equation 5.10, was used to analyze the *in vivo* digestibility.

$$Y = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{jjk} + c_l + \varepsilon_{ijkl} \quad (5.10)$$

where μ was the overall mean, α was the main effect of grain source, β was the main effect of fractionation using the TriQ, γ was the main effect of temperature on pelleting condition, $(\alpha\beta)_{ij}$ was the interaction between source of grain and fraction, $(\alpha\gamma)$ was the interaction between sources of grain and temperature of pelleting, $(\beta\gamma)$ was the interaction between fraction and temperature of pelleting $(\alpha\beta\gamma)_{jjlk}$ was the interaction between source fraction and temperature of pelleting, c_k with the random effect of the period, and ε_{ijlk} was the residual error.

5.4 Results

5.4.1 Rate of production.

More grain was sorted into the medium CP fraction, which also contained the outliers (Table 5.2; $P < 0.01$) and more barley was sorted into the LCP than into the HCP fraction ($P < 0.05$). The rate of production when sorting wheat (Table 5.2; $P < 0.01$) or barley (Table 5.2; $P < 0.01$) based on predicted CP differed between fractions. The CP (Table 5.3) and starch (Table 5.4) contents were similar between fractions for both wheat and barley ($P > 0.50$).

5.4.2 Water hydration capacity

Water hydration capacity (WHC) was similar among fractions ($P > 0.50$) but increased with conditioning temperature ($P < 0.01$) for both wheat and barley rations. Water hydration capacity of the rations prepared using HCP or LCP fractions of wheat or barley, sampled after the conditioner but before the pelleter, was unaffected by fraction ($P > 0.35$), but increased with increasing temperature (Table 5.5; $P < 0.01$). Similarly, the WHC of the pelleted feed was similar among fractions, but increased with increased temperature of the conditioner (Table 5.6; $P < 0.01$)

5.4.3 Viscosity

Diet viscosity was unaffected by either fraction or conditioner temperature (Table 5.7; $P > 0.10$).

5.4.4 C-peptide

C-peptide concentrations in blood samples obtained from pigs pre- or post-prandial was unaffected by treatment (Table 5.8; $P > 0.10$). There was no difference in C-peptide ($p$mol L^{-1}) between fractions before or after feeding ($P > 0.10$) or with temperature of pelleting ($P > 0.10$). No interaction between pelleting temperature and fraction was observed for either wheat or barley ($P > 0.10$). For wheat and barley, C-peptide for all treatments had an SEM greater than 10% of the average value.

5.4.5 Digestibility

For both wheat and barley-based rations, there was an interaction between conditioner temperature and fraction for apparent DMD (Table 5.11; $P < 0.05$). Total tract apparent DMD of the wheat-based ration was reduced by the high pelleting temperature, but only with the HCP fraction (Table 5.11; fraction x temperature, $P = 0.02$). Conversely, DMD was improved with increased pelleting temperature, but only with the LCP fraction (Table 5.11; fraction × temperature, $P = 0.01$).

The effects of sorting barley into fractions using an NIT seed sorter calibrated for CP content and high and low pelleting temperatures on standardized ileal AAD are described in Table 5.12, and for wheat in Table 5.13. Standardized ileal digestibility of Leu, Lys, Phe, and Val and all non-essential amino acid except Tyr increased when pigs were fed barley-based diets pelleted at a higher temperature and based on the HCP fraction (fraction x temperature interaction ($P < 0.01$). The SID of Tyr and ammonia increased with temperature when pigs were fed diets based on the HCP fraction but decreased with the LCP fraction (fraction × temperature interaction ($P < 0.01$). Conversely, the SID of His decreased when pigs were consuming the HCP diet pelleted at high temperature ($P < 0.05$). The SID of most of the amino acids for wheat-based rations were lower for the HCP diets. No interaction was observed for the SID of wheat-based ration for fraction × temperature (Table 5.13; $P \geq 0.05$). Under low temperature pelleting, the

SID of wheat-based rations was higher in AAD.

5.5 Discussion

Feed grade grains were purchased from multiple sources and incorporated into rations based on average nutrient content, ignoring the physical and chemical variations within the batch. Fractionating grain into subsamples based on specific chemical constituents has not been possible without destroying the grain during the analysis or reducing mill throughput. However, instruments are now commercially available which use NITS or NIRS technology to fractionate grain based on calibrations for specific traits (Walsh 2005; Slaughter 2009; Tønning et al. 2009). Attaining the full potential of such technology requires information about the differences between the fractions. We hypothesized that fractions sorted based on predicted CP content would differ chemically, that digestibility would differ between the fractions, and that fractions would interact differently with hydrothermal treatments, thus affecting digestibility.

The first objective of this experiment was to determine if the use of NIT technology would result in fractions differing in chemical traits which make a change in AAD. Secondly, we wished to determine if these fractions would respond differently to processing, specifically temperature, during pelleting. Even if such fractionation was possible, the method of processing for each fraction is still unclear. Initially, to identify if the equipment can be used commercially, the rate of fractions produced must be identified. The manufacturer has indicated that the rate of sorting is 10,000 kg h^{-1}, but no work has been published to verify this. The accurate determination of rate of sorting can determine the cost to the manufacturer and determine its viability within the commercial sector.

Production rate of the medium CP fraction was higher than either the low or the high CP fraction, and higher for wheat than for barley (Table 5.2; $P < 0.05$). This occurred because outliers are incorporated into the medium CP fraction. Reasons for outliers are not defined by the system and cannot be identified by the user. Pocket sizes around the drum of the TriQ are identical and thus variation in kernel size will result in some kernels being too small or too large for the pocket and these kernels will be identified as outliers. Additionally, the actual CP content of the kernel may not fall within the range of the CP content defined in the calibration.

Kernel size can impact processing (Slavin et al. 2000; Campbell et al. 2007; Warechowska 2014) and the TriQ will reduce this variability by using a drum which allows only similarly sized kernels to enter each fraction. The larger the wheat kernel, the higher the bulk density and the hardness index and ash content increase with lower particle sizes (Dziki and Laskowski 2004). Marshall et al. (1986a) investigated 11 cultivars of wheat and found that seed size and milling yield were positively correlated. Milling yield is defined as the percent of flour from a ton of wheat (Marshall et al. 1986a). The consistency in kernel size established using the same drum will result in a more predictable outcome in grinding quality and pelleting quality.

The TriQ uses NITS to predict the CP content of individual kernels; however, the fractions obtained in this experiment were similar in CP content (Table 5.3). The reasons for this are not known, as the TriQ has been validated by several other published reports (Tønning et al. 2009; Kautzman et al. 2015a; Kautzman et al. 2015b). The CP content of wheat (Delwiche 1995; Dowell et al. 2006) and wheat flour (Başlar and Ertugay 2011) have been predicted using NIRS with correlation coefficients greater than 0.85. It is not known if NIR predicts with higher accuracy and precision than NIT which is used by the TriQ.

The lack of an effect of fractionation on starch content (Table 5.4) or WHC (Table 5.5) was not surprising due to the lack of effect of fractionation on CP content. Protein and starch content in grains are negatively correlated (Hunt 1996). According to Berton et al. (2002), both the chemical composition of wheat and the processing conditions may impact WHC. For example, granular starch can absorb 39% to 87% of water by weight, while starch that has been damaged can absorb 200% to 430% (Berton et al. 2002). That the TriQ produced fractions that did not differ in starch or CP content means that WHC is likely to be similar between fractions unless they are processed differently (Table 5.5 and Table 5.6). The increase in WHC after pelleting (Table 5.5 and 5.6) can be attributed to exposure of the starch to heat. The WHC of the grain increased as the temperature increased from 25°C to 75°C (Toyokawa et al. 1989).

Water hydration capacity is correlated to viscosity and, therefore, the lack of an effect of fractionation on viscosity and an increase in viscosity with temperature was expected. Viscosity of wheat or barley can be affected by the gluten content (Butaki and Dronzek 1979) or by the

polysaccharide, β-glucan (Lamp et al. 2015). βeta-glucan, which occurs in the endosperm cell walls and aleurone cell walls of barley can increase the WHC which can cause an increase in viscosity (Bedford 1995; Li et al. 1996). Increased viscosity of the grain can result in reduced digestibility due to a reduction in the accessibility of the substrate as well as interference with absorption of nutrients (Knudsen et al. 2012). The increase in viscosity results in a lower rate of glucose entering the blood stream (Ou et al. 2001). Blood glucose level directly affects insulin production which is related to the C-peptide concentration (Heyduk et al. 2010). C-Peptide is directly related to pancreatic insulin but, compared to insulin, hepatic and prehepatic extraction is negligible (Brandenburg 2008; Regmi et al. 2010), thus plasma peptide C provides an estimation of insulin secreted by the pancreas. The fractions did not differ in viscosity and thus C-peptide concentration should not be different. C-Peptide in the plasma of pigs consuming the treatment diets was unaffected by fractionation of the grain and by pelleting.

The rate of starch digestion and absorption is dependent on the chemical characteristics of the starch and this affects insulin secretion (Regmi et al. 2010). The slow rate of starch absorption can cause a reduction in peak glucose absorption and thus a decline in the rate of C-peptide increase (Regmi et al. 2010). Starch that is high in amylose and has low *in vitro* digestibility results in a lower insulin level (Regmi et al. 2011). Starch digestion is dependent on several factors, including the structure of the starch, method of processing, animal age, feed intake, passage rate and absorptive capacity of the gut (Giuberti et al. 2014).

Pelleting typically improves feed efficiency (Lundblad et al. 2011) by reducing segregation of feed and increasing starch digestibility (Thomas et al. 1999). Steam conditioning for 50 seconds increased the soluble CP fraction, increasing the digestibility of CP content more than steam conditioning for 75 sec did (Huang et al. 2015).

The effect of processing temperature on AAD can be attributed to an increase in amino acid exposure to enzymatic activity; however, if processing temperature is higher, it could cause the amino acid(s) to become less accessible to enzymes due to Maillard reactions (van Rooijen et al. 2013). The interaction between temperature and fraction can be attributed to gelatinization of the starch (Lund and Lorenz 1984) increasing enzyme interactions with the substrate. At higher

processing temperatures, the reduction in digestibility was less than expected; this is most likely due to the temperature not being high enough to cause significant Maillard reaction to occur.

5.6 Conclusions

A commercial seed sorter that separates individual kernels of wheat or barley based on NITS predicted CP content was used to produce fractions for wheat and barley. However, the fractions produced were similar in CP and starch content. Despite this, apparent ileal DMD digestibility differed between fractions produced by the TriQ and processing temperature. These results demonstrated that while the NIT sorter did not provide fractions that differed in CP content, making the effect on digestibility difficult to explain, it could be due to statistical anomalies (Type I or Type II error). It is also possible that the fractions differed in other, unidentified, constituents. Future work is needed to determine why such fractions differed in digestibility when their major chemical constituents were similar.

5.7 Next stage

Further work is required to determine why fractions produced by the NIT seed sorted that were similar in starch and CP content differed slightly in digestibility when treatment diets were fed to pigs. For example, it is not known if non-structural polysaccharides and fibre content varied between fractions. Future work should compare wheat and barley varieties that vary in chemical composition. and identify if they can produce fractions that differ chemically.

5.8 Acknowledgements

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5.9 Conflict of interest

None

Table 5.1. The composition of the diets used in this study (% as fed).

Ingredients	Wheat based diet	Barley based diet	N free diet
Wheat[Z]	73.98	0.00	0.00
Barley[Y]	0.00	74.97	0.00
Soybean meal	21.73	19.53	0.00
Canola meal	0.00	0.00	38.39
Lysine HCl	0.32	0.30	0.00
L-threonine	0.13	0.10	0.00
DL-methionine	0.04	0.07	0.00
Choline chloride	0.00	0.00	0.05
Dextrose (sugar)	0.00	0.00	10.00
Corn starch	0.00	0.00	40.51
Canola oil	1.67	2.67	3.00
Limestone	1.03	0.93	0.50
Calcium phosphate (Biofos)	0.60	0.93	1.90
Salt	0.30	0.30	0.40
Vitamin Micro	0.00	0.00	0.15
Mineral Micro	0.00	0.00	0.10
Mineral[X]	0.10	0.10	0.00
Vitamin[V]	0.10	0.10	0.00
Potassium carbonate	0.00	0.00	0.40
Magnesium oxide	0.00	0.00	0.10
Solka floc	0.00	0.00	4.00
Celite[U]	0.40	0.40	0.40
Titanium oxide	0.10	0.10	0.10

Note: Two different mineral premixes were used as the nitrogen free diet was produced after the fifth period of the metabolic trial and the manufacturer that produced the initial mineral had stopped producing feed.

[Z]Wheat was fractionated into three fractions based on predicted CP content where only the low CP and high CP was used to produce two different diets using one of the fractions.

[Y]Barley was fractionated into three fractions based on predicted CP content where only the low CP and high CP was used to produce two different diets using one of the fractions.

[X]Zinc sulphate 100 mg/kg; Ferrous sulphate 80 mg/kg; Copper sulphate 50 mg/kg; Manganous sulphate 25 mg/kg, Calcium iodate 0.50 mg/kg and Sodium Selenite 0.10 mg/kg.

[V]Vitamin A 8250 IU/kg; Vitamin D 825 IU/kg; Vitamin E 40 IU/kg; niacin 35 mg/kg; D-pantothenic
acid 15 mg/kg; menadione 4 mg/kg; folacin 2 mg/kg; thiamine 1 mg/kg; D-biotin 0.2 mg/kg; Vitamin B_{12} 25 ug/kg.

[U]Celite® 545, Celite Corporation, Lompoc CA, USA.

Table 5.2. Average production rate for fractions of wheat or barley based on predicted crude protein obtained using the BoMill TriQ single kernel near infrared transmittance spectra.

		Fraction				
		HCP	LCP	Medium CP	SEM[z]	*P value*
Production rate (kg/second)	Wheat	13.41a	14.78a	27.59b	1.091	*<0.01*
	Barley	10.40a	13.96b	18.55c	0.510	*<0.01*

[z]SEM: Standard error of the mean.

Table 5.3. Average CP content of wheat and barley grain fractionated by TriQ into two fractions based on predicted CP, (high crude protein (HCP), low crude protein (LCP) and the initial unsorted (UNS) grain).

	Fraction				
	HCP	LCP	UNS	SEM[z]	*P value*
Wheat	14.97	14.46	14.65	2.721	*0.99*
Barley	11.46	11.14	11.01	0.321	*0.65*

[z]SEM: standard error of the mean.

Table 5.4. Average starch content of wheat and barley grain fractionated by TriQ into two fractions based on predicted CP, (high crude protein (HCP), low crude protein (LCP) and the initial unsorted (UNS) grain).

	Fraction (%)				
	HCP	LCP	UNS	SEM[z]	*P value*
Wheat	54.22	56.45	56.49	3.197	*0.86*
Barley	53.06	52.24	53.40	1.305	*0.82*

[z]SEM: standard error of the mean.

Table 5.5. Water hydration capacity for complete mixed rations after pelleting that maximizes inclusion of wheat or barley sorted into fractions based on predicted CP using a near infrared transmittance spectroscopy.

	Water hydration capacity (%)			
	Wheat	SEM[z]	Barley	SEM
HCP	60.13	0.385	60.57	0.484
LCP	59.92		60.25	
P-value	*0.69*		*0.65*	
25°C[y]	43.86		46.92	
65°C[x]	65.42	0.471	64.13	0.591
75°C[w]	70.80		70.18	
P-value	*0.01*		*0.01*	

Note: Fraction x temperature was P > 0.05.
[z]SEM: Standard error of the mean.
[y]25 is the temperature in degree Celsius the sample was exposed to for 30 minutes.
[x]65 is the temperature in degree Celsius the sample was exposed to for 30 minutes.
[w]75 is the temperature in degree Celsius the sample was exposed to for 30 minutes.

Table 5.6. Water hydration capacity for complete mixed rations prior to pelleting that maximizes inclusion of wheat or barley sorted into fractions using near infrared transmittance.

	Water hydration capacity (%)			
	Wheat	SEM[z]	Barley	SEM
HCP	58.06	0.366	58.58	0.336
LCP	58.25		58.94	
P-value	*0.71*		*0.45*	
25°C[y]	46.88	0.450	54.43	0.411
65°C[x]	60.55		57.90	
75°C[v]	67.04		63.90	
P-value	*0.01*		*0.01*	

Note: Fraction x temperature was P > 0.05.
[z]SEM: Standard error of the mean.
[y]25 is the temperature in degree Celsius the sample was exposed to for 30 minutes.
[x]65 is the temperature in degree Celsius the sample was exposed to for 30 minutes.
[v]75 is the temperature in degree Celsius the sample was exposed to for 30 minutes.

Table 5.7. Viscosity measurement in centipoise (mPa. s) for sixteen diets composed of wheat or barley. Grains were sorted by near infrared transmittance spectroscopy to produce low crude protein (LCP) and high crude protein (HCP) fractions.

	Viscosity (mPa. s)			
	Wheat	SEM[z]	Barley	SEM
HCP	1.70		2.25	
LC[x]	1.67	0.121	1.64	0.432
P-value	*0.86*		*0.32*	
LT[y]	1.81		1.56	
HT[x]	1.56	0.121	2.33	0.432
P-value	*0.16*		*0.21*	

Note: Fraction x temperature was P > 0.05.
[z]SEM: Standard error of the mean.
[x]LT: Low temperature represents the temperature that was set at the conditioner to be lower than 70°C.
[y]HT: High temperature represents the temperature that was set at the conditioner to be higher than 85°C.

Table 5.8. C-peptide measurement in pmol/L for sixteen diets composed of wheat or barley fed to cannulated pigs. Grains were sorted with a BoMill TriQ to produce either a low crude protein (LCP) or a high crude protein (HCP) fraction. Each diet contained one of these fractions and was formulated to maximize grain inclusion. Blood samples were collected 30 minutes before feeding and 45 minutes after feeding.

	C-peptide (*p*mol/L)			
	Wheat	SEM[Z]	Barley	SEM
HCP	305.98	39.790	265.97	39.123
LCP	230.93		327.96	
P-value	*0.19*		*1.00*	
LT[y]	220.80	39.768	296.93	39.123
HT[x]	316.12		297.01	
P-value	*0.10*		*0.27*	

Note: Fraction x temperature was P > 0.05.
[z]SEM: Standard error of the mean.
[y]LT: Low temperature represents the temperature set at the conditioner to be lower than 70°C.
[x]HT: High temperature represents the temperature set at the conditioner to be higher than 85°C.

Table 5.9. Calculated nutrient composition of wheat-based rations fed to cannulated pigs. Wheat was sorted with a BoMill TriQ to produce either a low crude protein (LCP) and a high crude protein (HCP) fraction. Each diet contained one of these fractions and was formulated to maximize grain inclusion. Digestibility was determined and corrected using the marker titanium oxide.

	Dietary treatments							
Source	1				2			
Fraction[Z]	LCP		HCP		Low		High	
Temperature[Y]	Low	High	Low	High	Low	High	Low	High
DM (%)[X]	90.25	89.76	90.98	89.38	89.66	90.07	89.32	88.74
CP(%)	19.76	19.76	20.27	19.45	23.49	24.69	25.41	25.85
Ca (%)	1.51	1.49	1.90	1.78	1.6	1.63	1.60	1.60
Phos (%)	0.59	0.59	0.62	0.6	0.7	0.70	0.74	0.74
Na (%)	0.16	0.17	0.17	0.19	0.21	0.21	0.22	0.23
ADF (%)	3.15	4.14	3.14	3.19	3.43	4.06	3.95	4.25
NDF (%)	9.39	10.50	8.47	7.03	11.96	9.48	10.30	11.74
Histidine (%)	0.51	0.51	0.52	0.50	0.513	0.51	0.52	0.50
Isoleucine (%)	0.64	0.62	0.66	0.63	0.636	0.62	0.66	0.63
Leucine (%)	1.28	1.25	1.28	1.25	1.28	1.25	1.28	1.25
Lysine (%)	1.06	1.04	1.13	1.09	1.056	1.04	1.13	1.09
Methionine (%)	0.30	0.32	0.31	0.29	0.297	0.32	0.31	0.29
Phenylalanine (%)	0.84	0.83	0.84	0.90	0.838	0.83	0.84	0.90
Threonine (%)	0.80	0.79	0.82	0.81	0.802	0.79	0.82	0.81
Valine (%)	0.74	0.71	0.75	0.73	0.736	0.71	0.75	0.73
Alanine (%)	0.82	0.74	0.75	0.76	0.821	0.74	0.75	0.76
Ammonia (%)	0.42	0.42	0.44	0.44	0.42	0.42	0.44	0.44
Arginine (%)	1.13	1.06	1.06	1.05	1.132	1.06	1.06	1.05
Aspartic (%)	1.63	1.60	1.67	1.62	1.634	1.60	1.67	1.62
Cysteine (%)	0.28	0.28	0.30	0.27	0.275	0.28	0.30	0.27
Glycine (%)	0.75	0.78	0.79	0.80	0.747	0.78	0.79	0.80
Glutamic acid (%)	4.27	4.22	4.335	4.31	4.269	4.22	4.34	4.31
Serine (%)	0.91	0.92	0.922	0.91	0.914	0.92	0.92	0.91
Tyrosine (%)	0.49	0.50	0.516	0.65	0.494	0.50	0.52	0.65
Proline (%)	1.32	1.29	1.296	1.30	1.315	1.29	1.30	1.30

[Z]Fraction produced by the TriQ.
[Y]Temperature exposed to the feed entering the conditioner (low temperature pelleting at 70°C versus high temperature pelleting at 85°C).
[X]Dry matter in percent.

Table 5.10. Calculated nutrient composition of barley-based rations fed to cannulated pigs. Barley was sorted with a BoMill TriQ to produce a low crude protein (LCP) and a high crude protein (HCP) fraction. Each diet contained one of these fractions and was formulated to maximize grain inclusion.

	Dietary treatments							
Source	1				2			
Fraction[Z]	Low		High		Low		High	
Temperature[Y]	Low	High	Low	High	Low	High	Low	High
DM (%)[X]	91.27	87.94	86.22	89.52	90.75	89.76	90.65	89.61
CP (%)	17.54	18.01	19.85	19.80	17.72	18.44	18.32	18.37
Ca (%)	3.13	2.59	1.73	1.79	1.24	1.44	1.40	1.42
Phos (%)	0.58	0.64	0.69	0.75	0.67	0.71	0.69	0.66
Na (%)	0.13	0.14	0.16	0.17	0.14	0.16	0.15	0.15
ADF (%)	4.60	6.50	6.64	6.18	7.80	8.08	6.76	9.90
NDF (%)	12.84	14.72	16.85	17.15	18.11	20.82	18.37	19.36
Histidine (%)	0.38	0.50	0.461	0.47	0.38	0.496	0.46	0.47
Isoleucine (%)	0.62	0.69	0.727	0.64	0.62	0.693	0.73	0.64
Leucine (%)	1.02	1.24	1.235	1.18	1.02	1.242	1.24	1.18
Lysine (%)	0.76	1.17	1.030	1.04	0.76	1.167	1.03	1.04
Methionine (%)	0.27	0.31	0.295	0.30	0.27	0.306	0.30	0.30
Phenylalanine (%)	0.67	0.93	0.810	0.78	0.67	0.933	0.81	0.78
Threonine (%)	0.59	0.85	0.773	0.74	0.59	0.850	0.77	0.74
Valine (%)	0.59	0.76	0.696	0.72	0.59	0.764	0.70	0.72
Alanine (%)	0.57	0.74	0.694	0.73	0.57	0.737	0.69	0.73
Arginine (%)	0.66	1.21	1.099	1.00	0.66	1.207	1.10	1.00
Aspartic (%)	1.18	1.66	1.512	1.46	1.18	1.664	1.51	1.46
Cysteine (%)	0.23	0.27	0.249	0.28	0.23	0.270	0.25	0.28
Glycine (%)	0.60	0.76	0.712	0.72	0.60	0.757	0.71	0.72
Glutamic acid (%)	2.98	3.79	3.654	3.67	2.98	3.791	3.65	3.67
Serine (%)	0.66	0.86	0.838	0.80	0.66	0.860	0.84	0.80
Tyrosine (%)	0.39	0.59	0.463	0.39	0.39	0.589	0.46	0.39
Proline (%)	1.08	1.30	1.403	1.33	1.08	1.302	1.40	1.33

[Z]Fraction produced by the TriQ.
[Y]Temperature exposed to the feed entering the conditioner (low temperature pelleting at 70°C versus high temperature pelleting at 85°C).
[X]Percentage dry matter.

Table 5.11. Percent apparent dry matter digestibility for sixteen diets composed of wheat or barley fed to cannulated pigs. Grains were sorted with a BoMill TriQ to produce a low CP (LCP) and a high CP (HCP) fraction. Each diet contained one of these fractions and was formulated to maximize grain inclusion. Digestibility was determined and corrected using the marker titanium oxide

	Percent digestibility			
	Wheat	SEM[z]	Barley	SEM
HCP	74.55	0.762	70.71	1.424
LCP	83.01		70.34	
P-value	*0.01*		*0.86*	
LT[y]	80.74a	0.762	67.18b	1.424
HT[x]	76.82b		73.90a	
P-value	*0.01*		*0.02*	
LT x HCP	78.29b	0.969	70.91ab	2.088
HT x HCP	70.81c		70.52ab	
LT x LCP	83.20a	1.169	63.44b	1.937
HT x LCP	82.83a		77.29a	
P-value	*0.02*		*0.01*	

[z]SEM: Standard error of the mean.
[y]LT: Low temperature represents the temperature set at the conditioner to be lower than 70°C.
[x]HT: High temperature represents the temperature set at the conditioner to be higher than 85°C.

Table 5.12. Standardized ileal amino acid coefficient digestibility for predicted low crude protein (LCP) and predicted high crude protein (HCP) barley-based diets processed at low and high pelleting temperatures fed to cannulated pigs. Barley fractions were obtained by running two sources of barley through the BoMill TriQ to produce LCP and HCP fractions.

	Fraction			Pelleting temperature				HCP		LCP			
	LCP	HCP	*P-value*	LT	HT	*P-value*	SEM[Z]	LT[Y]	HT[X]	LT	HT	SEM	*P-value*
Histidine	1.25	1.67	*0.74*	1.25	1.16	*0.01*	0.020	1.36a	1.15b	1.15b	1.18b	0.025	*0.02*
Isoleucine	0.87	0.87	*0.83*	0.87	0.87	*0.58*	0.007	0.85ab	0.86a	0.89a	0.85b	0.010	*0.01*
Leucine	0.84	0.85	*0.74*	0.84	0.85	*0.13*	0.007	0.81c	0.87a	0.86ab	0.83bc	0.010	*0.01*
Lysine	0.89	0.88	*0.22*	0.88	0.89	*0.13*	0.006	0.86b	0.91a	0.89ab	0.87b	0.009	*0.01*
Methionine	0.90	0.89	*0.22*	0.89	0.90	*0.32*	0.008	0.88ab	0.92a	0.90ab	0.88b	0.010	*0.01*
Phenylalanine	0.84	0.84	*0.60*	0.83	0.85	*0.04*	0.008	0.79c	0.88a	0.86ab	0.83bc	0.012	*0.01*
Threonine	0.90	0.92	*0.04*	0.91	0.91	*0.98*	0.007	0.91	0.93	0.91	0.89	0.010	*0.10*
Valine	0.84	0.84	*0.82*	0.83	0.85	*0.07*	0.009	0.80b	0.87a	0.85ab	0.83ab	0.013	*0.01*
Alanine	0.78	0.77	*0.66*	0.75	0.79	*0.04*	0.012	0.73b	0.82a	0.78ab	0.76ab	0.012	*0.01*
Arginine	0.89	0.91	*0.03*	0.89	0.91	*0.01*	0.005	0.85c	0.93a	0.92ab	0.90b	0.008	*0.01*
Aspartic acid	0.80	0.83	*0.28*	0.81	0.82	*0.01*	0.007	0.76c	0.86a	0.84ab	0.81b	0.010	*0.01*
Cysteine	0.81	0.82	*0.60*	0.80	0.84	*0.06*	0.013	0.77b	0.85a	0.82ab	0.82ab	0.019	*0.05*
Glycine	0.73	0.78	*0.01*	0.73	0.78	*0.01*	0.012	0.66b	0.79a	0.79a	0.77a	0.007	*0.01*
Glutamic acid	0.86	0.88	*0.02*	0.86	0.88	*0.01*	0.005	0.82b	0.89a	0.89a	0.86a	0.007	*0.01*
Serine	0.83	0.84	*0.52*	0.82	0.85	*0.03*	0.008	0.79c	0.88a	0.86ab	0.83bc	0.012	*0.01*
Tyrosine	0.87	0.85	*0.06*	0.86	0.87	*0.34*	0.009	0.83bc	0.91a	0.88ab	0.82c	0.012	*0.01*
Proline	0.79	0.86	*0.05*	0.80	0.84	*0.17*	0.023	0.73b	0.84ab	0.87a	0.84ab	0.032	*0.04*
Ammonia	0.80	0.81	*0.76*	0.80	0.81	*0.76*	0.014	0.77a	0.83b	0.83b	0.78a	0.020	*0.01*

a,b,c,d Values within the same row without a common superscript are significantly different ($P < 0.05$).

[Z]SEM: Standard error of the mean.

[Y]LT: Low temperature represents the temperature set at the conditioner to be lower than 70°C.

[X]HT: High temperature represents the temperature set at the conditioner to be higher than 85°C.

Table 5.13. Standardized ileal amino acid coefficient digestibility for predicted low crude protein (LCP) and predicted high crude protein (HCP) wheat-based diets processed at low and high pelleting temperatures fed to cannulated pigs. Fractions were obtained by running two sources of wheat through the BoMill TriQ to produce LCP and HCP fractions.

	Fraction			Pelleting temperature			
	HCP	LCP	*P-value*	LT[Z]	HT[Y]	SEM[X]	*P-value*
Histidine	1.04	1.04	*0.93*	1.04	1.05	0.010	*0.63*
Isoleucine	0.93	0.93	*0.66*	0.94	0.92	0.005	*0.01*
Leucine	0.92	0.93	*0.02*	0.93	0.92	0.003	*0.01*
Lysine	0.95	0.95	*0.28*	0.96	0.94	0.003	*0.01*
Methionine	0.96	0.95	*0.04*	0.96	0.95	0.050	*0.03*
Phenylalanine	0.92	0.94	*0.01*	0.93	0.93	0.003	*0.28*
Threonine	0.94	0.95	*0.01*	0.95	0.94	0.002	*0.01*
Valine	0.91	0.91	*0.45*	0.92	0.90	0.007	*0.02*
Alanine	0.90	0.90	*0.91*	0.91	0.89	0.004	*0.01*
Arginine	0.96	0.95	*0.35*	0.96	0.95	0.004	*0.01*
Aspartic acid	0.90	0.91	*0.39*	0.92	0.89	0.006	*0.01*
Cysteine	0.90	0.91	*0.35*	0.91	0.90	0.008	*0.10*
Glycine	0.87	0.89	*0.12*	0.89	0.87	0.011	*0.24*
Glutamic acid	0.96	0.96	*0.71*	0.96	0.95	0.004	*0.09*
Serine	0.92	0.93	*0.10*	0.93	0.92	0.003	*0.01*
Tyrosine	0.93	0.95	*0.02*	0.95	0.94	0.005	*0.15*
Proline	0.96	0.96	*0.41*	0.96	0.96	0.095	*0.95*
Ammonia	0.91	0.92	*0.46*	0.93	0.91	0.007	*0.05*

Note: Fraction x temperature was P > 0.05.
[Z]SEM: Standard error of the mean.
[Y]LT: Low temperature represents the temperature set at the conditioner to be lower than 70°C.
[X]HT: High temperature represents the temperature set at the conditioner to be higher than 85°C.

6 GENERAL DISCUSSION

The objectives of this research were to identify if sorting wheat and barley on a single kernel basis based on predicted CP content would produce fractions that were physically and chemically different. It was predicted that fractions would differ in grinding characteristics (e.g. DGM) and respond differently to hydrothermal treatment, resulting in differences in viscosity, water hydration capacity and fermentation, or digestibility affecting nutrient digestibility in ruminants and monogastrics.

6.1 Fractions produced by the BoMill TriQ

The fractions produced by the TriQ did not differ in CP or starch content for any of the studies. This could be because calibrations did not reflect the chemical composition for the grain tested. Near infrared reflectance spectroscopy has been used previously to predict CP content with a high degree of accuracy and precision (Delwiche 1998; Delwiche and Hruschka 2000; Lin et al. 2014). The calibration of the TriQ could not be tested as it is proprietary in nature. In addition, CP content variability within a source may not have been enough to be detected by the calibration of the TriQ. Future work should test this calibration by using wheat and/or barley with extremes of CP content.

The TriQ sorts on an individual kernel basis using a drum with pores of specific dimensions allowing individual kernels to be sorted if they meet this specific dimension. Both wheat (Dziki and Laskowski 2004; Baasandorj et al. 2015) and barley (Baasandorj et al. 2015) vary in kernel dimensions. The large variation in kernel size could be another factor that may have contributed to the large medium CP fraction. To reduce this variation, grain should initially be sieved to match the dimensions of the pores within the drum, which could potentially reduce the variability in CP content and reduce the proportion of kernels that are outliers. The value of presorting based on kernel size must be determined and further investigated. Additionally,

multiple drums could be used to resort the medium CP fraction, allowing kernels of different sizes to be sorted.

6.2 Chemical and physical variation

Variations in both physical and chemical characteristics of grain have been reported by several authors (Kong et al. 1995; Cai et al. 2013; Shewry et al. 2013). Grains can vary between and within varieties due to growing location, environmental conditions, agriculture practices and post-harvest handling (Daniel and Triboi 2000; Mladenov et al. 2001; Li et al. 2016). Sorting of grain to produce fractions that are more consistent in chemical or physical characteristics could provide several benefits. For example, reduced variation in kernel size resulted in more consistent grinding and more homogeneous chemical composition (Dziki and Laskowski 2004). This has the potential to mazimize nutrient efficiency.

However, in the current study, starch and CP content were similar between fractions obtained after sorting using a single kernel NIT sorter. This suggests that fibre content would also be similar, but fibre was not measured, and small differences in digestibility could be potentially explained by differences in fibre content or could be due to statistical anomalies. Zijlstra et al. (1999) demonstrated that nonstructural polysaccharide variation within wheat can affect DM digestibility.

There were numerical, but not statistically significant, differences between fractions from independent wheat or barley in physical characteristics (Chapter 3). This lack of consistency within a fraction could reduce the benefit of sorting. The lack of homogeneity in grain size within a fraction could explain the standard deviation of DGM when the different fractions were ground (Table 4.4 and Table 4.6). Dziki and Laskowski (2004) indicated that kernel size had the largest effect on particle size following grinding. Moreover, they showed that the energy consumption for grinding is dependent on particle size as indicated by Equation 6.1:

$$E = K\left(\frac{1}{\sqrt{d}} - \frac{1}{\sqrt{D}}\right) \quad (6.1)$$

where E is the total specific milling energy and D and d represent the particle size of the product before and after milling, respectively. Additionally, Fang et al. (1998) illustrated that particle size of the final product was affected by the initial surface area of the product entering the roller mill. This indicates that if kernels vary in size, there will be greater variation in particle size following grinding.

The variation in kernel dimensions could affect the consistency of the grinding characteristics. If the variation between loads is different, DGM and standard deviation of DGM will be different. For agricultural products that are ground using either a hammer mill or a roller mill, a variety of particle sizes and shapes is produced. This results in increased scattering when the energy source from an NIR beam strikes a sample, and thus developing calibrations will require mathematical corrections. The large amount of grain entering the medium CP fraction could be attributed to the sensitivity of NIRS to variation in particle size. Sensitivity of NIRS to particle size has been addressed by several authors (Isaksson and Næs 1988; Dhanoa et al. 1994).

6.3 Digestibility

The digestibility of each fraction was investigated using monogastric and ruminant models. The change in digestibility or lack thereof could be due to the minimal chemical and physical variation observed between fractions. Expected differences in digestibility attributed to the processing conditions, particle reduction and hydrothermal treatment were observed.

Grinding barley or wheat with a hammer mill or a roller mill resulted in a reduction in particle size and this resulted in changes in digestibility, regardless of the fraction. A reduction in particle size is necessary to achieve maximum digestibility. Dry matter and CP content digestibility following roller milling were lower than after hammer milling, probably due to a lower proportion of fines (particles less than 1 mm) entering the final product. Surface area contributes greatly to digestibility and will increase when the fines increase which could explain the results for hammer milling.

There were significant variations in apparent ileal DMD (Table 5.11) of wheat observed in the swine trial. This cannot be attributed to the CP fraction or starch content of the fractions, as they were similar, but it may have been a result of changes in amino acid or fibre type, or it could be a statistical anomaly; a replication of the experiment would be needed to determine the cause. Variation in amino acid can result in changes in hardness (Jenner et al. 1991; Giroux and Morris 1997) which could impact post grinding characteristics. Fibre content can vary between wheat sources (Shewry and Hey 2015). Barley did not show differences in apparent ileal DMD for monogastrics but did show differences in SID. The reason for this could not be identified, but further work will be needed as this was not observed between fractions. Wheat did not show differences in SID, which indicated that digestibility was more likely affected by fiber types. A possible reason for the observed differences in DMD or lack of difference in SID for the wheat rations could be a Type I or Type II error but it could not be determined in this study.

6.4 The strengths and limitations of this study

6.4.1 Strengths

The current study investigated a commercial NIT sorter that could, in theory, separate individual grains based on CP content. The strength of this study was that five different feed grade sources were purchased and used for both wheat and barley. Additionally, these sources were assessed for grinding under both hammer and roller milling to identify if fractions would differ in particle size and particle size distribution under different grinding parameters. These fractions also were exposed to different processing temperatures during the conditioning phase of feed production. Each fraction was also assessed using monogastric and ruminant models of ileal digestibility and total gas production, respectively. These findings could provide an overview of how each fraction could be utilized in the agriculture industry.

6.4.2 Weaknesses

The findings of no significant difference in CP and starch content between fractions may be due to a lack of difference within the sources examined. Additionally, a more comprehensive understanding of the variation in chemical composition within a fraction was not investigated.

These limitations require further investigation to determine the cause of the variations between the fractions. To solve this problem, initial sources of grain should be purchased that differ in CP content. These sources should be mixed at different proportions and then sorted to evaluate the ability of the equipment to sort by CP CONTENT. These fractions also should be assessed for their complete chemical profiles as well as post grinding and hydrothermal characteristics. This would give a complete profile of each fraction and assessment of the value of using a NITS sorter. Additionally, a complete economic analysis of the use of a sorter and how the grade of each fractions would change is necessary.

6.5 Conclusions

The objectives of this research were to identify if sorting on a single kernel basis for both wheat and barley based on predicted CP content would produce fractions that were physically and chemically different, but the findings indicated that protein and starch content did not differ between fractions produced using a commercial NIT sorter. These differences had been predicted to result in differences in grinding characteristics (e.g. DGM) and the lack of difference in chemical composition resulted in no difference in grinding characteristics. These fractions were not differentially affected by hydrothermal treatment. Feeding trials with pigs revealed that DM digestibility of wheat and amino acid digestibility of barley were affected by the predicted CP of fractions. However, no differences in digestibility were observed for fermentation using an *in vitro* model of rumen fermentation. In conclusion, the use of a single kernel NIT sorter did not produce fractions that were different in CP content or starch content and thus its value in the feed industry cannot be guaranteed even though DMD differed. Additionally, rate of sorting for wheat differed from barley, and thus the value of such equipment may depend on the type of cereal.

6.6 Future research recommendations

A. Future research is required to assess different types of sorting equipment by examining their capability to separate grain and the consistency of the products under different conditions, such as:
    a. varieties of grain

b. quality of grains

B. Future research is needed to analyze the benefits of increased grain chemical and physical homogeneity on animal performance and how this consistency will impact grinding and hydrothermal treatment outcomes.
C. Future research needs to examine how animal feed should be formulated when using a more homogeneous ingredient, as current models are based on averages of a load.
D. Future research needs to examine the economic benefit of using sorting equipment and how it may affect future feeds if it became more relevant in the food industry.

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APPENDIX A: FEED INTAKE

The total amount of feed consumed per pig and all pigs was calculated prior to the production of the feed. The amount of feed consumed per treatment per pig and all pigs was indicated in Table A.1. An average weight of 70 kg for each pig was used for the calculations. Feed was provided at 3 times the maintenance requirement. The calculations are indicated below:

Maintenance requirement	$= 110 \times (70 \text{ kg body weight})^{0.75}$
Total daily calorie allowance	= 3 × (Maintenance requirement)
Total daily feed allowance	= Total daily calorie allowance/DE content of the feed
Feed needed per 12 hours	= Total daily feed allowance / 2
Total feed needed for 1 period	= 9 × Total daily feed allowance
Addition of feed for post-surgery	= 5 + Total feed needed

To ensure enough feed would be available, 250 kg of feed per treatment was produced.

Table A.1. Amount of feed allocated for each pig during this study period

	Barley ration	Wheat ration
Average weight of pig during study (kg)	70	70
Maintenance requirement (kcal)	2662	2662
Feed allowance (above maintenance)	3	3
Total daily calorie allowance (kcal)	7986	7986
DE content of the diet (kcal)	3282	3534
Total daily feed allowance (kg)	2433	2260
Feed allowance per 12 hours (kg)	1217	1130
Feed allowance for nine days (kg)	21897	20340
Feed allowance for six pigs (kg)	131382	122040
Feed allowance with surgery adaptation (kg)	145 kg	135 kg

APPENDIX B: WATER HYDRATION CAPACITY

The WHC for three sources of feed grade wheat obtained from three different locations within Saskatchewan had significant variation in their hydration capacity ($P = 0.041$) as indicated in Figure B.1. The WHC was lowest when temperature of the wheat was at 21°C and highest at 75°C. According to Figure B.1, unground grain for all sources was lower for its respective temperature for WHC.

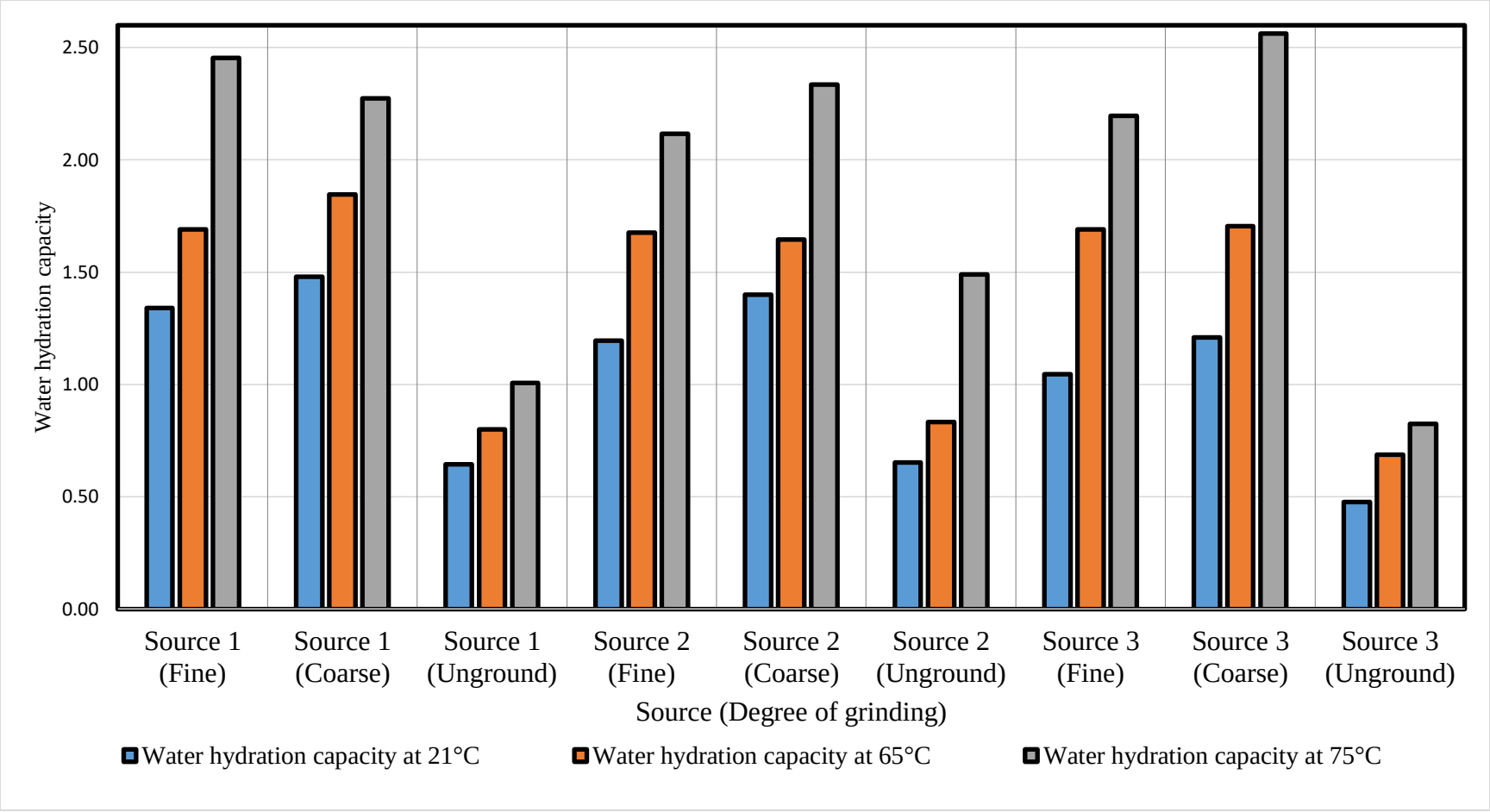

Figure B.1. The water hydration capacity of feed grade wheat obtained from three different locations within Saskatchewan and processed to three different particle sizes (unground, finely ground and coarsely ground) with a hammer